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U.S. Department of Health and Human Services

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16. Abstract (Limit: 200 words)

A survey of workers exposed to carbon-disulfide (75150) (CS2) at concentrations below the current standard of 20 parts per million (ppm) was conducted. The cohort consisted of 146 male workers at a rayon staple factory (SIC-2823). The comparison group consisted of 233 workers at the same facility having no CS2 exposure. The average ages of the cohort and comparisons were 38.2 and 33.9 years, respectively. Subjects were administered comprehensive medical examinations that included neurological, endocrine, metabolic, reproductive, and cardiovascular evaluations. Industrial hygiene sampling for CS2 was performed. CS2 concentrations ranged from 0.58 to 12.64ppm. Exposed workers showed very little increased morbidity, but exposure dependent increases in pathological changes such as increased frequency of angina and myocardial infarction, systolic and diastolic blood velocity, increased symptoms of muscular weakness, increased low density of lipoproteins, increased fasting blood sugar, increased proportion of abnormal sperm forms, and increased incidence of retinal abnormalities. The authors conclude that the present standard of 20ppm leaves little margin of safety. Additional research on the cardiovascular toxicity of CS2 and review of the current standard are recommended.

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EXECUTIVE SUMMARY AND CONCLUSIONS

A cross-sectional medical and industrial hygeine survey of 146 workers exposed to carbon disulfide and 233 generally similar unexposed workers at the same plant site was performed to assess the effect of occupational exposure to CS_2 at levels below the present U.S. occupational standard of 20 ppm (8-hour TWA). Based on effects of carbnon disulfide reported in various previous studies, tests were chosen to assess cardiovascular, neurological, psychological, endocrine, metabolic, and reproductive status. Most of the variables were examined both by stratifying workers by present exposure levels (DL = less than 3 ppm; M = 3 to 7.1 ppm; DH = greater than 7.1 ppm) and by regression on log(exposure index), where exposure index (exp. ind.) is the sum of the historic exposure level for each month of a worker's tenure at the plant. Exposed and control groups differed in age, smoking history, and education; these variables were used to adjust means or as covariates in regression analyses where there seemed to be a potential for confounding.

The results showed intergroup differences in a number of physiological, behavioral, or anatomic variables; several variables, shown abnormal in other groups of exposed workers, were not significantly different in this study; a few showed differences in a direction opposite that expected. The differences by strata were generally small, although often statistically significant at the alpha = 0.05 level. Regression on log(exp. ind.)

generally explained less than 5% of the variance, although the correlation was often statistically significant. The urinary iodine-azide test, a screening procedure used to estimate absorption of CS_2, was found to be an unreliable index to exposure in the range below 20 ppm.

Table ES-1 is a summary listing of the tests and outcomes (for details, the reader is referred to individual chapters of the report). "Acute" refers to results obtained by grouping subjects into definitely low (DL = less than 3 ppm), medium (M = 3 to 7.1 ppm), definitely high (DH = more than 7.1 ppm), and control groups based on work assignment at the time of the study. "Chronic" refers to results obtained by reference to the exposure index. Direction (Dir.) was scored "+" if the group of all exposed workers had a higher numerical score or proportion with the attribute, "-" if the exposed workers had a lower score or percentage, and "0" if the groups were equal. Gradient (Grad.) was scored "+" if there was an increasing gradient of response with increasing exposure, "-" if the response decreased with exposure, and "0" if the response varied irregularly or did not change with exposure level. NOTE: Gradient and direction are scored without respect to alpha level; significance (Sig.) is noted in the third column and relates to an alpha of 0.05 for the "Direction" comparison only. This table is intended to provide a composite overview and to highlight relationships likely to be obscured by fragmented inspection of the individual results. The expected direction of difference (Exp.) was based on previous reports or on hypotheses that CS_2 will have adverse effects related to reported effects.

Examination of comparisons based on present exposure levels for the 52 tests whose outcomes were "predictable," based on an assumption of CS_2 toxicity in this work force qualitatively similar to reported effects showed:

- 36 of the tests showed differences between exposed and controls in the direction expected; 13 of the 36 were statistically significant;
- only one test of the 52 was significantly different in the direction opposite that expected.

This type of analysis involves multiple significance testing; about 3 tests should be "significant" by chance alone. The number found significant exceeds 3 by a substantial margin, suggesting that the results are not explained as a factitious result of multiple significance testing. Since the tests are not mutually independent, statistical testing of the signs is not appropriate and caution must be used in drawing inferences such as the one above.

A major purpose of this study, to find or estimate the limits of safe exposure, has at least been approached. In the population studied, present levels of exposure cause very little excess morbidity but are associated with a wide range of pathological changes.* In view of divergent foreign reports,[1-10] the qualifying phrase "in the population studied" assumes special importance. Excess mortality due to cardiovascular disease among Finnish workers and striking excesses in diabetes and retinal pathology among Japanese workers exposed to CS_2 at levels near the present U.S. Standard is cause for concern over margin of safety; the increase in retinal microangiopathy in this study heightens that concern. The consensus opinion of the principal investigators is that the present standard (20 ppm) provides little or no safety margin, even in this predominantly Anglo-Saxon workforce.

We conclude:

1. There is a need for additional work on the cardiovascular toxicity of CS_2 as suggested by the dose-response patterns of the ocular findings in this study and by the variation in prevalence of microaneurysms and hemorrhages among workers with similar exposures in different countries.
2. The demonstration of pathology in a study population that had been exposed to levels of CS_2 at and below the present standard suggests that the current standard needs review.

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* The changes alluded to here include: increased frequency of angina and myocardial infarction; systolic and diastolic blood pressure; increased heart rate, increased low density lipoproteins and LDL/HDL ratio; ulnar nerve, increased distal and residual latency; peroneal nerve, increased distal and residual latency; sural nerve, inadequate action potential and decreased sensory conduction velocity; increased symptoms of muscular weakness, blurred vision, trembling hands, memory difficulty, numbness or tingling, dizziness, insomnia, fatigue, headaches, numbness, and decreased libido; increased choice reaction time; longer eye hand coordination times and increased eye hand variance; increased fasting blood sugar; increased proportion of abnormal sperm forms; ulnar nerve, decreased amplitude ratio and motor conduction velocity; peroneal nerve, decreased amplitude ratio and motor conduction velocity; lower scores on the Profile of Mood States test; and increased frequency of retinal abnormalities. As noted, not all of these are statistically significant.

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Table ES-1: Summary of results obtained in testing

Test	Acute			Chronic		
	Grad.[1]	Dir.[2]	Sig.[3]	Dir.	Sig.	Exp.[4]
Demographics (Chapter 5)						
- Age (Years)	+	+	+	+	+	0
- Length of Employment (Years)	+	+	+	+	+	0
- Exposure Index	+	+	+	(Ident.)		+
- Smoking (ever/ex/non)	-	-	-			0
- Smoking (packs/year)	-	-	+			0
- Smoking (pack-years)	0	0	0			0
- Drinking	0	-	-			0
- Education	0	-	-			0
- GGTP	+	-	-			0
- SGOT	0	-	-			0
- SGPT	0	+	-			0
Rose questionnaire (Chapter 6)						
- Angina	0	+	0	0	0	+
- Myocardial infarction	0	+	0	0	0	+
Blood pressure[5] (Chapter 6)						
- systolic	+	+	+	+*	+	+
- diastolic (IV)	+	+	- (p=.06)	+*	-	+
- diastolic (V)	+	+	-	+*	-	+
EKG (Chapter 6) (Abnormal)	+	+	-	-	-	+
STI (Chapter 6)						
- QS_2	0	-	-	+*	-	
- LVET	0	-	-	+*	-	-
- PEP	0	-	-	+*	-	+
- PEP/LVET	0	0	-	-*	-	+

1. Grad. = + if there is an increasing gradient of response with increasing exposure, - if response decreases with exposure, 0 if response varies irregularly or does not change with exposure level.
2. Dir. = + if response is higher for all exposed than for controls, - if response is lower, 0 if response is the same.
3. Sig. = + if significant at 0.05 level, 0 if too few observations to consider, - if not significant at 0.05 level.
4. Exp. = Expected Dir., based on previous reports or on hypotheses that CS_2 will have adverse effects related to reported effects.
5. Adjusted for age.

* Joint effect of log(exp. ind.) and age·log(exp. ind.) causes effect shown after several years of life; at an earlier age, the sign is opposite that given.

Table ES-1: Summary of Results Obtained in Testing (cont'd.)

Test	Acute			Chronic		Exp.
	Grad.	Dir.	Sig.	Dir.	Sig.	
Hemoglobin (Chapter 6)	+	0	+	Not tested		0
Heart rate (Chapter 6)	+	+	+	-*	+	
Blood lipids (Chapter 6)						
- Cholesterol[5]	+	-	-	-*	+	+
- Triglycerides[5]	-	-	-	-*	+	0
- HDL[5]	+	+	-	+*	-	0?
- LDL[5]	+	+	-	-*	+	+
- LDL/HDL[5]	0	+	-	-*	+	+
- MANCOVA: Cholesterol, Triglycerides, HDL, LDL					+	
Blood Lead (Chapter 6 & 8)	+	+	+	Not tested		0
Zn (serum) (Chapter 6)	0	+	-	Not tested		-
Cu (serum) (Chapter 6)	0	+	-	Not tested		-
Zn (urine) (Chapter 6)	-	+	-	Not tested		-
Retinal microaneurysms (Chapter 7)						
(Abnormal)	+	+	+	+	+	+
Retinal hemorrhages (Chapter 7)						
(Abnormal)	-	+	+	+	+	+
Nerve conduction tests (Chapter 8)						
- Ulnar - Distal Latency	0	+	-	Not tested		+
- Ulnar - Residual Latency	0	+	-	Not tested		+
- Ulnar - Amplitude Ratio	-	-	-	Not tested		+
- Ulnar - Motor Cond. Velocity	0	-	-	-	+	-
- Peroneal - Distal Latency	-	+	-	Not tested		+
- Peroneal - Residual Latency	-	+	-	Not tested		+
- Peroneal - Amplitude Ratio	-	-	+	Not tested		+
- Peroneal - Motor Cond. Velocity	-	-	+	-	+	-
- Peroneal - No action potential	+	-	- (p=.07)	Not tested		+
- Sural - Latency	0	0	-	Not tested		+
- Sural - Amplitude	+	0	-	Not tested		-
- Sural - Sensory Cond. Velocity	0	-	+	Not tested		-
- Sural - No action potential	0	+	+	Not tested		+

Table ES-1: Summary of Results Obtained in Testing (cont'd.)

	Acute			Chronic		
Test	Grad.	Dir.	Sig.	Dir.	Sig.	Exp.
Symptoms (Chapter 9)						
- Muscular weakness	0	+	-	Not tested		+
- Blurred vision	+	+	+	Not tested		+
- Trembling hands	0	+	+	Not tested		+
- Memory difficulty	0	+	+	Not tested		+
- Walking difficulty	-	-	-	Not tested		+
- Numbness or tingling	+	+	-	Not tested		+
- Dizziness	0	+	+	Not tested		+
- Fatigue	0	+	+	Not tested		+
- Headaches	0	+	-	Not tested		+
- Insomnia	0	+	+	Not tested		+
- Numbness	+	+	-	Not tested		+
- Mental condition	0	0	-	Not tested		+
- Decreased Appetite for Sex	+	+	-	Not tested		
- Difficulty with erection	0	-	-	Not tested		+
Psychological and psychomotor tests (Chapter 9)						
$POMS_1$	0	-	-	Not tested		-
MMPI	-	+	-	Not tested		
Neisser	0	-	+	Not tested		
Memory	-	-	-	Not tested		
Choice reaction time	0	+	-	Not tested		+
Simple reaction time	+	-	-	Not tested		+
Eye hand	0	+	-	Not tested		+
Eye hand variance	-	+	-	Not tested		+
Depth perception	+		-	Not tested		
Visual acuity			-	Not tested		
Endocrine tests (Chapter 10)						
T_4 by RIA	+	-	-	+*	-	
T_3 Uptake	+	-	+	-*	-	
T_7 Index	+	-	-	-*	-	
MANCOVA: T_4, T_3, T_7			+		-	
Fasting Blood Sugar (FBS)	-	+	-	+*	-	+
Semen analyses (Chapter 11)						
Semen Volume (cc)	-	+	-	Not tested		0
Sperm Count	0	+	-	Not tested		-
Abnormal Forms	0	+	-	Not tested		+

Chapter 1 - INTRODUCTION

A. HISTORICAL BACKGROUND

Carbon disulfide (CS_2) was discovered by the German chemist Lampadius in 1796.[1] Since then, it has found widespread use in many industrial applications but has produced unwanted effects in some humans exposed to it. Its use as an anesthetic gas by Simpson in the 1840's caused hallucinations, headache and nausea.[2] Its use in the India-Rubber industry, common in the mid 1850's, caused loss of "... will power ... loss of memory ... [and] self-contempt".[3] With the onset of large-scale rubber production in the early 20th century, symptoms of psychosis were reported. The British Journal, Dangerous Trades, described a factory in which the windows of the vulcanizing room had to be barred to keep CS_2 intoxicated workers from leaping out during attacks of mania.[4] With the development of the viscose rayon industry, various other symptoms of carbon disulfide intoxication were reported; these included tingling and numbness of the extremities, weakness of limbs, loss of appetite, weight loss, headache, sexual dysfunction, impaired vision, and gastrointestinal disturbances.[5,6] A survey of the viscose rayon industry published by the Pennsylvania Department of Labor and Industry in 1938 found that the viscose rayon workers complained of "... poor exercise tolerance, marked fatigue on slight exertion, palpitation, shortness of breath, precordial and substernal pain, and faintness".[3] The concept of carbon disulfide toxicity as a multisystem disorder evolved from these early descriptions of carbon disulfide intoxication.

In May, 1977, the National Institute for Occupational Safety and Health (NIOSH) published Criteria for a Recommended Standard ... Occupational Exposure to Carbon Disulfide.[7] NIOSH recommended "that carbon disulfide concentration in workplace air not exceed 3 mg/M^3 or 1 part per million (ppm) as a 10-hour TWA (time weighted average) concentration during a 40-hour week." This represented a considerable change from the previous (and current) OSHA standard of 20 ppm. In 1982, the American Council of Governmental Industrial Hygeinists (ACGIH) adopted a TLV of 10 ppm.

NIOSH's proposed standard was based on several human studies which suggested that carbon disulfide affects the cardiovascular system, the nervous system, the retinal vessels, and the reproductive system. Some of these studies were adequate but dealt with carbon disulfide exposures much higher than are usually seen in modern American workplaces. Other studies were incomplete in either design, exposure data, or statistical analysis. Since a suitable standard could not be determined from the available literature, a comprehensive medical study of an American worker population exposed to CS_2 in concentrations at or below the current standard of 20 ppm was planned and conducted in the spring of 1979.

B. PURPOSE

The purpose of this cross-sectional medical and industrial hygiene study was to determine the health effects of occupational exposure to CS_2 for at least one working year at a documented concentration approximating the current Federal exposure limit of 20 ppm.

C. SPECIFIC AIM

The specific aim of this study was to compare groups exposed to carbon disulfide with an unexposed comparison group with respect to the following health variables:

1. Cardiovascular Status
    - Blood Pressure
    - History of Coronary Heart Disease
    - Serum Lipid Values
    - Electrocardiogram
    - Systolic Time Intervals
    - Retinal Pathology
2. Neurological and Psychological Status
    - General Visual Function
    - Psychological Examinations
    - Nerve Conduction Velocity
    - Psychomotor Examinations
3. Endocrine and Metabolic Status
    - Serum Thyroxin Levels
    - Serum Trace Metal Levels (Zn, Cu)
    - Iodine Azide Reaction
    - Serum Glucose Values
4. Reproductive Status
    - Sperm Count and Morphology
    - Reproductive history

This composite report describes the methods and results for all of the above areas except the reproductive history.

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CHAPTER 2 - STUDY DESIGN

A. GENERAL

This study was designed as a cross-sectional medical survey in combination with an industrial hygiene survey of carbon disulfide exposure. Using both medical and industrial hygiene data, dose-response effects of subject exposure to CS_2 were determined. Two important sources of potential bias should be noted. First, only those workers healthy enough to have remained on the job (i.e., workers actively employed) were available for participation in the survey. Second, only those workers willing to consent to the examinations actually participated in the survey; a higher proportion of exposed workers than comparison workers consented. These two potential sources of bias are inherent in all cross-sectional medical surveys.

B. SELECTION OF STUDY GROUP

After meetings with industry and labor representatives and visits to the four U.S. rayon staple plants, a factory in northeastern Tennessee was chosen as the best available study site (vide infra). Rayon staple is a product like cotton wool, used in such products as disposable diapers and sanitary napkins. The plant was opened in 1948 for production of rayon filament (thread or yarn). A viscose rayon staple plant was opened at the same site in 1956; a nylon filament plant in 1963, a polyester filament plant followed in 1966; and a nylon-polyester staple plant in 1967. The rayon filament plant ceased production in 1974. The chronology of plant operations is summarized in Figure 2-1.

Records of environmental area samples at the viscose plant dated from 1957 and typically showed air concentrations of carbon disulfide vapor between 1.5 ppm and 60 ppm. Levels are generally lower than those noted in Finland[1] and Japan[2] (see Figures 2-2, 2-3, and 2-4). The highest levels were area samples and were taken at locations where a problem was thought possible. Since workers did not ordinarily remain long in these areas, we think actual worker exposure was nearer the 20 ppm level desired for the study, but we cannot exclude the possibility that some workers were exposed to higher levels.

Since few women were employed at the plant, the study was restricted to males. A non-exposed (comparison) group, employed by the same company and generally similar to the exposed group, was available at the same site. Eligibility for inclusion in the exposed or comparison group was determined from individual work histories obtained from the company before the study. Workers who had been employed in the viscose rayon staple plant for at least one year and who were currently employed there were eligible for inclusion in the exposed group; their prior employment could have included the old rayon filament plant or any of the other synthetic plants listed above.

The comparison group was derived from the polyester filament, nylon filament, and the nylon-polyester staple plants, where there was virtually no occupational exposure to carbon disulfide (general environmental levels on the plant site were 0.2 ppm). Those included in the comparison group had to have been employed at least one year in one or more of these plants. Workers from these areas with previous experience in either the rayon staple or filament plant were excluded from the comparison group.

A number of job types, including general maintenance, yard work, etc., were excluded from both exposed and comparison groups because their work would take them into areas both with and without carbon disulfide exposure. A few people in this category were inadvertently included in the medical tests but were thereafter excluded from statistical analysis.

C. METHODS

1. General Procedures

Prior to the study, the investigating team met with workers in small groups, explained the purpose of the study and the nature of the tests, and invited all eligible workers to participate. At the time of the study, the employer made space in the administrative area of the plant available for testing. A NIOSH trailer was parked nearby, and used for registration and check-out. The cafeteria area was used during off-hours for blood drawing.

At the start of the actual screening, the employee reported to the trailer, where he was checked in and given a semen collection jar if he had volunteered for that portion of the study. He then went next door and upstairs to the testing area. Psychological tests were given first, followed by psychomotor tests, and tests of visual acuity and depth perception. Mydriatic drops were instilled and, during a planned delay to allow full dilation of thje pupils, the medical history questionnaire was administered by a trained interviewer. The employee was then examined by direct ophthalmoscopy and photographed with the Topcon Retinal Camera. He next moved to a room where a brief physical examination was performed; from there to the blood pressure, ECG, and systolic time interval tests; and finally to the nerve conduction area.

Blood drawing was done en masse at several dates during the three-week course of the study.

2. Medical Tests Performed

A computer-generated random study number was assigned to each volunteer. Scheduling was done by study number, within constraints of current shift assignment, so that cases and controls were mixed. No information on exposure history was displayed on forms available to examiners, and examiners were prohibited from asking questions or engaging in conversation which would yield this information. The tests done included the following:

a. Cardiovascular Tests Performed

(1) Hypertension

Blood pressure was determined after a 15 minute rest period during which the worker was in a supine position. With the worker still supine, the pressure was measured twice in the right arm (unless an anatomic defect forced use of the left arm). A random-zero sphygmomanometer was used, to minimize observer error, and all meaurements were taken by the same technician. The random-zero sphygmomanometer is a device which adds or subtracts a random amount from the pressure reading. The amount of the change is not known until after completion of the measurement, so a technician will not unconsciously bias the results toward, for example, even numbers.[3]

(2) Coronary Heart Disease

A resting 12-lead electrocardiogram (ECG) was done on each subject. Each ECG was read by three cardiologists and reported using the Minnesota Code.[4]

(3) Impaired Cardiac Contractility

Systolic time intervals were determined using instruments which simultaneously recorded ECG, heart sounds at the cardiac apex, and carotid artery pulse wave. The systolic time interval (STI or QS_2) is measured from onset of the QRS complex to the closing of the aortic valve. The time from onset of the QRS to opening of the aortic valve constitutes the isovolumetric contraction time or pre-ejection phase (PEP) and the time from aortic opening to aortic closure constituting the left ventricular ejection time (LVET).[5,6]

(4) Retinal Microaneurysms

Each subject underwent pupillary dilation with a short acting mydriatic. After dilation was complete, direct ophthalmoscopy was performed and the results recorded. Each subject then had two pictures taken of each retina using a Topcon retinal camera, a monochromatic light source, and panchromatic film. The film was later processed and mounted as black and white slides identified only by a randomly-assigned code number. Each slide was read by an ophthalmologist. Results were reported separately for each subject's right and left eye as either 1) normal, 2) having definite or uncertain microaneurysms, or 3) having definite or uncertain hemorrhages.

3. Psychiatric Tests Performed

Each participant was tested with the Profile of Mood States (POMS)[7] and with the mania scale drawn from the Minnesota Multiphasic Personality Inventory (MMPI)[8]. Taken together, these scales measure each worker along six identifiable mood or affective states (tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, confusion-bewilderment) and for mania.

4. Neuropsychological Tests Performed

a. Peripheral Neuropathies

Peroneal motor and sural sensory nerve conduction velocities and associated muscle action or nerve action potentials were measured. When time permitted, ulnar motor conduction velocity and muscle action potentials were determined. Latencies were calculated for motor nerves. Skin temperature was measured for each subject and used to adjust the nerve conduction velocities.[9]

b. Psychomotor Losses

Two tests were used to measure psychomotor function:

(1) The Visual Choice-Reaction Time Test required the subject to move his index finger from a central button to one of eight buttons lighted in a random sequence. The time to move off the central button measured decision time (simple reaction time), while the time from release of the central button to depression of the lighted button is one measure of motor function (complex reaction time).

(2) The Michigan Hand-Eye Coordination test required the subject to move a stylus through 119 holes in a plate. Test scores were the mean time to move the stylus from hole to hole and the variability in the interhole movement time.

c. Visual Function Alterations

Visual function was measured using two tests:

(1) The Neisser Test measured the rate of visual search by asking the subject to look for target letters on a sheet of paper containing rows of random arrangements of letters.

(2) Static visual acuity, depth perception, and peripheral vision were measured by standard methods, using a Bausch and Lomb(tm) Orthorater(tm).

d. Memory:

In the forward digit-span test, the subject viewed a series of up to eight digits on a video display screen and was required to recite them in the same order shown.

5. Endocrine and Metabolic Tests Performed

a. Hyperglycemia

The fasting blood sugar was measured.

b. Hypercholesterolemia

Total cholesterol, triglycerides, low and high density lipoproteins were measured with the subject in a fasting state.

c. Thyroid disorders

Thyroxin (T_4) and triiodothyronine (T_3) levels were determined on the fasting blood specimens.

d. Trace metal depletion

Zn, Cu, and total serum protein were determined on the blood samples.

6. Reproductive Tests Performed

Sperm count and morphology were determined on the subset of the cohort who were willing to volunteer. Volunteers were asked to abstain from sexual activity for at least 48 hours before collection. Specimens were produced by masturbation, collected in a sterile container, and brought to the examination center. Color and consistency of the sample were noted. The specimen was frozen and transported to the University of Cincinnati for analysis.

7. Physical Examination

In order to rule out conditions which might affect other tests, a brief physical examination was provided. Heart murmurs and testicular abnormalities, in particular, were sought.

8. Blood tests for possible confounding variables

a. Gamma glutamyl transpeptidase (GGTP) (plus SGOT and SGPT, if GGTP abnormal) was assayed to rule out liver disease which might indicate chronic excess intake of alcohol; alcoholism might affect the outcome of neuropsychological tests.

b. Hemoglobin was checked to rule out anemia as a cause of tachycardia which might, in turn, affect systolic ejection time.

c. Blood lead was determined since an elevated body lead burden might affect the outcome of neuropsychological tests.

9. Urinary iodine-azide test for worker monitoring

The urinary iodine-azide reaction has been widely used as an index of environmental exposure to CS_2; it was performed on a subset of workers and controls to determine the test's adequacy for this purpose. The urinary iodine-azide reaction was found to be an unreliable index to exposure to CS_2 in the range below 20 ppm, even with the use of such refinements as correction for creatinine excretion, prolonging the observation time for the reaction time from three to eight hours, use of the E index of Vasak[10], or correcting for body surface. For results of these analyses, the reader is referred to a Master's thesis titled "The Use of the Iodine-Azide test on Urine of Workers Exposed to Levels of Carbon Disulfide At or Below the Current [US] Federal Standard" submitted by Marcel-André Boillat, M.D. to the University of Cincinnati November, 1979; a summary of the work was also published.[11].

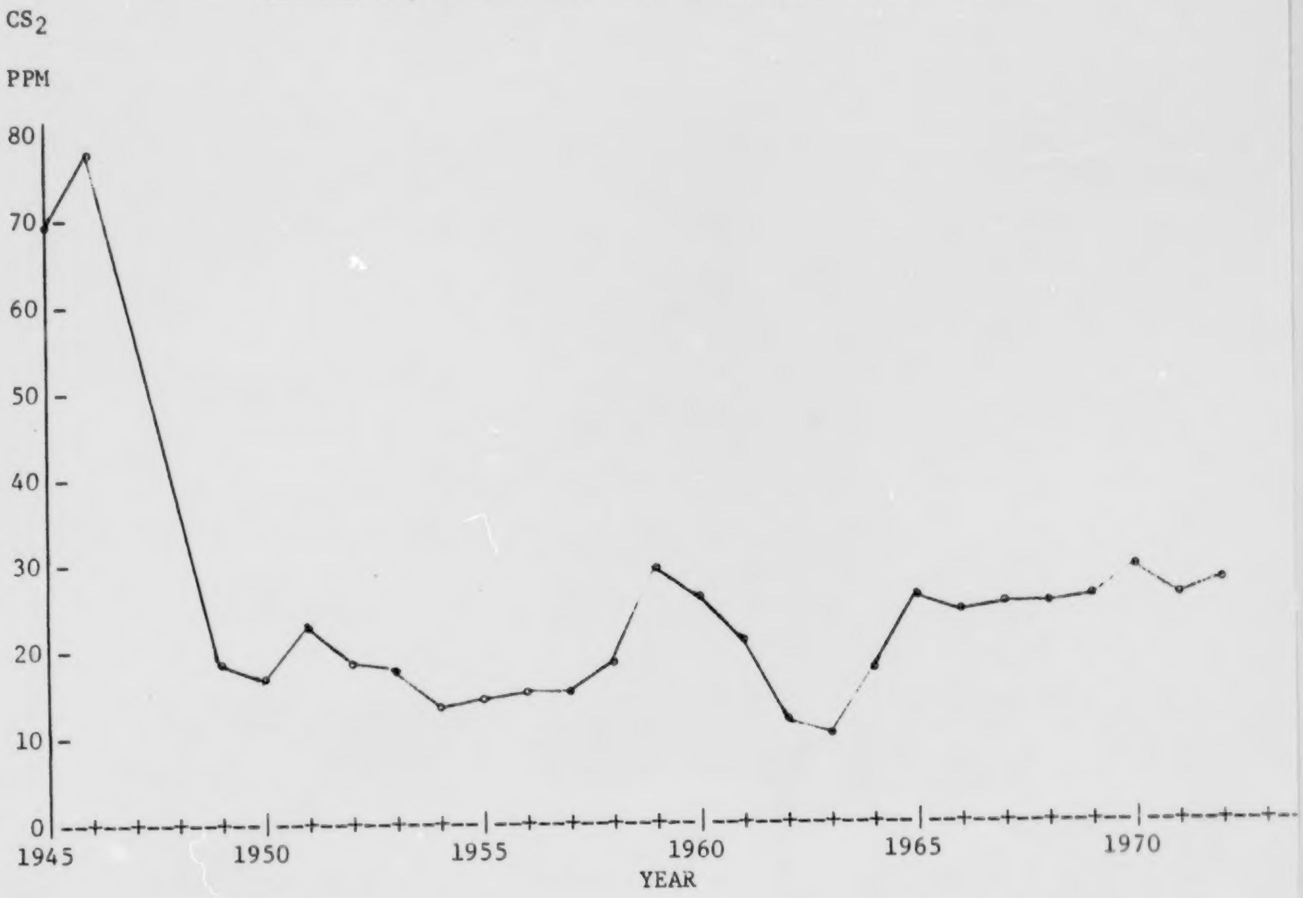
Figure 2-1

CHRONOLOGY OF PLANT ADDITIONS
AT A SITE IN
NORTHEASTERN TENNESSEE

```
 1955           1960           1965           1970           1975           1980
--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|-

(Opened 1945)
------------------------RAYON FILAMENT------------------------|

     |-----------------------------RAYON STAPLE-----------------------------------

                          |-----------------NYLON FILAMENT------------------------

                                   |------------POLYESTER FILAMENT----------------

                                      |--------NYLON-POLYESTER STAPLE--------------

--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|--+--+--+--+--|-
 1955           1960           1965           1970           1975           1980
```

Figure 2-2

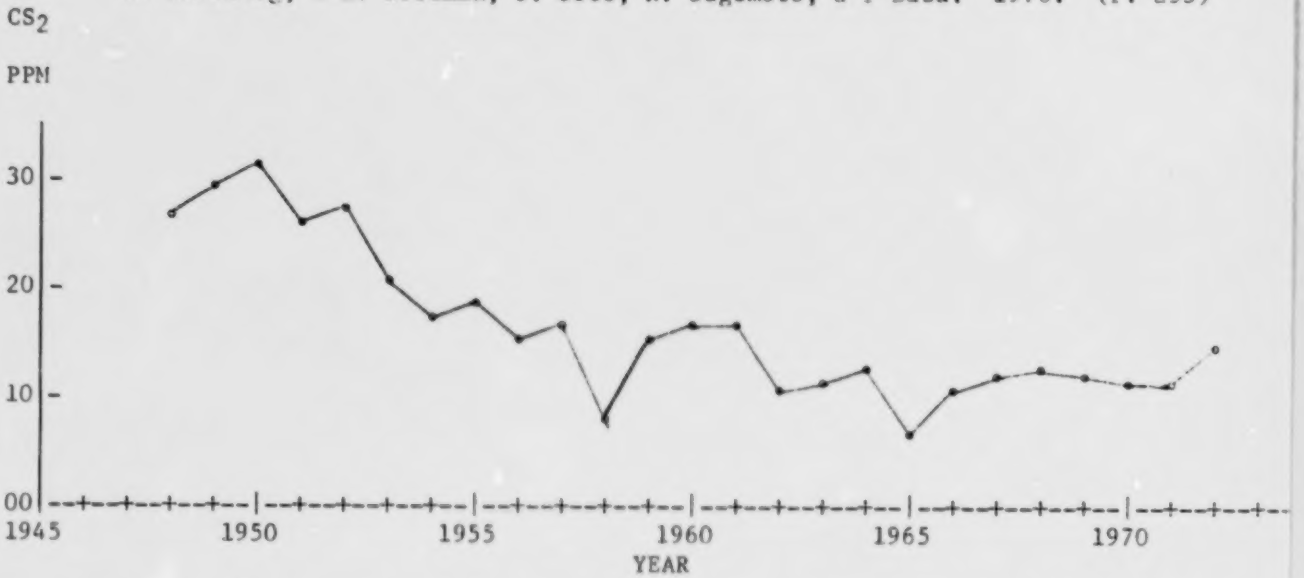
Environmental levels of CS_2 in the Finnish rayon plant studied by Ch. Raitta, M. Tolonen, & M. Nurminen. 1974. (P. 153)



Levels shown are approximately those of the spinning department, which had higher levels than the xanthation room and viscose ripening room, but lower than the spinning bath room.

Figure 2-3

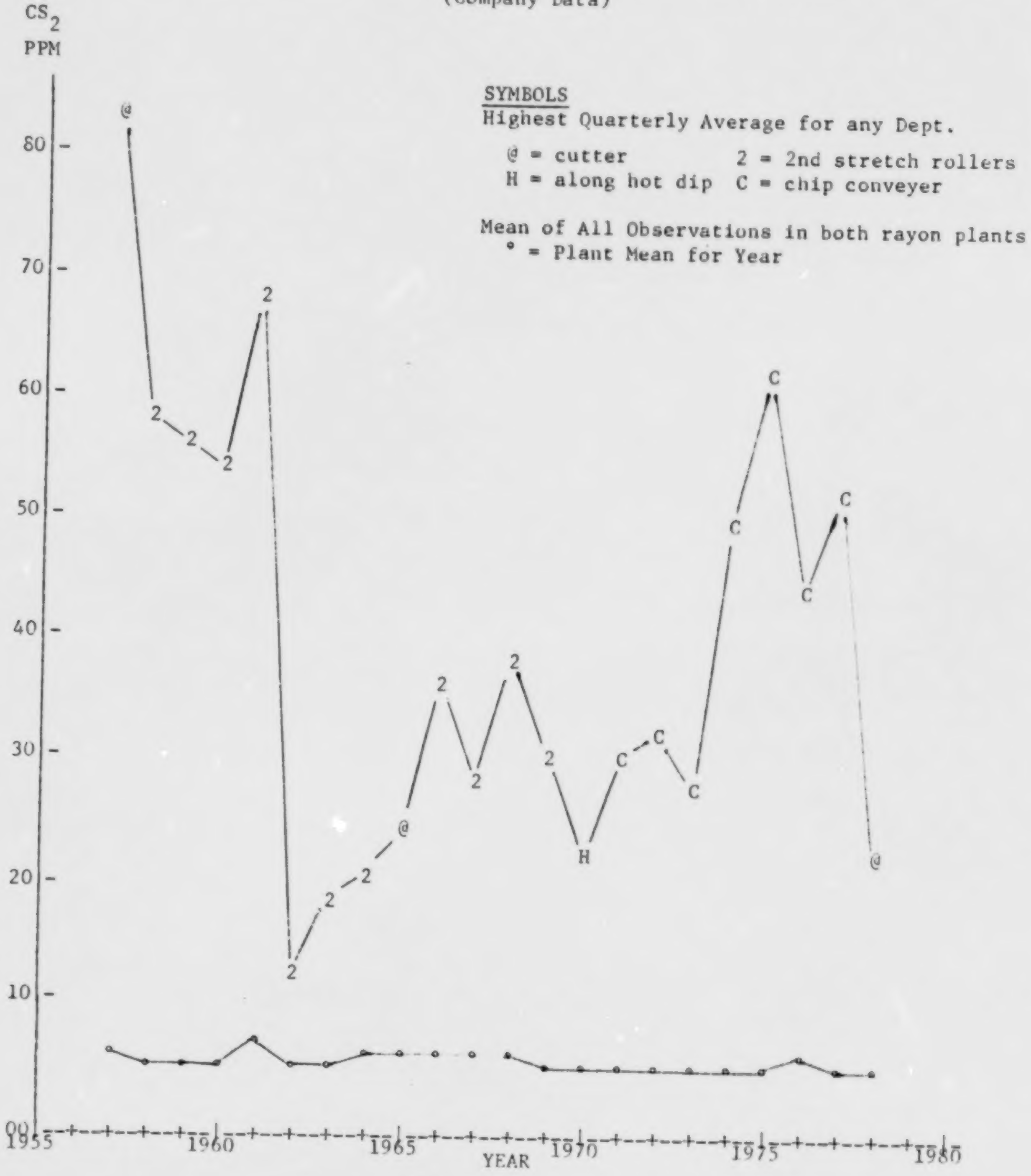
Environmental levels of CS_2 in the Japanese rayon plant studied by M. Tolonen, S. Hernberg, C-H. Nordman, S. Goto, K. Sugimoto, & T Baba. 1976. (P. 253)



Values above correspond approximately to the levels for "2nd room" as given in the paper. This room had, in general, the highest levels of the three, but is still no much different from the others.

Figure 2-4

Levels of CS_2 in the Study Plant by Year
Plant Mean and Highest Quarterly Average for any Department
(Company Data)



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E. SUMMARY

To determine dose-response effects of human exposure to CS_2, a cross-sectional medical survey was carried out in combination with an industrial hygiene survey of workers in a viscose rayon plant. The plant studied was chosen for evaluation because environmental samples of CS_2 vapor dating from 1957 showed air concentrations of carbon disulfide between 1.5 ppm and 60 ppm, with most observations below the current OSHA standard of 20 ppm. A comparison group was derived from nylon and polyester plants at the same site. Workers who volunteered for the study were examined on the plant premises.

Studies performed included: semen analysis; psychological tests; psychomotor tests; tests of visual acuity, function, and depth perception; a medical history questionnaire; direct ophthalmoscopy and retinal photography; a brief physical examination; blood pressure determination; electrocardiogram; systolic time interval measurement; and nerve conduction tests. Blood tests included: fasting blood sugar; total cholesterol; triglycerides; low and high density lipoproteins; thyroxin (T_4) and triiodothyronine (T_3) levels; Pb; Zn; Cu; total serum protein; gamma glutamyl transpeptidase (GGTP) (with SGOT and SGPT if GGTP abnormal); and hemoglobin. Urinary iodine-azide reaction was determined for a subset of workers and controls.

CHAPTER 3 - INDUSTRIAL HYGIENE

A. THE RAYON STAPLE PLANT

The rayon staple plant is an enclosed multi-level building which accepts raw materials and converts them into bales of rayon staple (Figure 3-1). On the top floor, at the beginning of the process, sheets of cellulose pulp are weighed, then placed in a soaking press containing caustic soda. When the cellulose has softened, the excess lye solution is squeezed out of the cellulose pulp by a press (Step 1). The pulp is then placed in a shredding machine which produces crumbs of the cellulose (Step 2). The crumbs are aged in a temperature-controlled room (Step 3). After aging, the crumbs are placed in a "churn" where they are mixed with liquid carbon disulfide (Step 4). This mixture is sent through a chute to a dissolving tank containing dilute caustic soda (Step 5). Here the crumbs are transformed into a viscose solution with the consistency of honey. The viscose solution is transported through pipes to another room for filtration and aging under vacuum (Step 6). After aging, the viscose is transported by pipes to the spinning room. The spinning room contains numerous troughs of dilute sulfuric acid. Each trough has many pipes with attached thimble-like platinum cups called spinnerets protruding into them. Each spinneret is perforated with 10 to 10,000 small holes. Viscose traveling from the aging room goes to the spinnerets and is forced through the fine holes into the acid bath (Step 7). This process coagulates the viscose to cellulose strands and releases some carbon disulfide vapor. The strands are gathered together to form a tow (Step 8) which is passed to the cutter where "acid chips" of rayon staple are made (Step 9). The chips pass over a conveyor belt for washing and more carbon disulfide is removed from the product (Step 10). Next the staple is placed in a dryer where the previously wet product becomes fluffy and white (Step 11). Staple is then passed to the baler and the finished bales are stored for shipment (Step 12).

Historical exposures to carbon disulfide vapor have usually varied from "very small" to 15-20 parts per million, depending on the area under consideration. Table 3-1 shows the job types for which area environmental sample data of CS_2 were available from company industrial hygiene records. Excursions above 20 ppm have occurred in all time periods, but were more common before 1965. The environmental area samples generally overestimate the worker exposure. They were taken only at locations which the hygienist thought might present a potential for overexposure and often, as in the cases of the chip conveyer and at the cutter, the worker typically spent very little time at or near the sample point. We cannot be certain that workers were never exposed to 8-hour time-weighted-average levels of 20 ppm or higher but think this was not common, if it did occur.

B. CURRENT EXPOSURES: CARBON DISULFIDE EXPOSED WORKERS

Exposed workers were divided into three groups on the basis of their current job title: definitely high (DH), medium or moderate (M), and definitely low (DL). The classification was established before beginning the field phase of the study and was the consensus of the company and NIOSH industrial hygienists, based on a knowledge of the process and the historical sampling data. The DH group was ultimately found to have CS_2 exposure greater than 7.1 ppm; the M group exposure was between 3 and 7.1 ppm; while the DL group had less than 3 ppm exposure at the time of the NIOSH study.

Most of the rayon staple workers are assigned to specific jobs which they perform every day. Each of these jobs fits into one of the steps of the process as described above. Occupational exposure to CS_2 was determined by attaching a personal air sampler (with charcoal tube) to numerous workers in each job category. Samples were obtained during a routine 8-hour shift. The collection of personal environmental samples was performed on 12 days at two different times (March and April). After collection, the samples were frozen and at a later date shipped to the laboratory for analysis.

Analysis of air samples was completed by desorbing the charcoal tubes in 2 ml of benzene in 2 ml vials overnight. A $28^1/_2$" column packed with 5 percent OV-210 on chromosorb W-HP was used at 25°C to separate the carbon disulfide from the benzene. A flame photometric detector was used to detect the carbon disulfide. Standards were prepared using SKC lot 107 charcoal.

Table 3-2 shows the number of air samples taken for each job category, the mean value for each category and the standard deviation of values for each. It can be seen that CS_2 exposures found in this study varied from less than 1 to about 16 ppm.

In addition to carbon disulfide exposure, rayon staple workers had potential exposure to hydrogen sulfide (H_2S), tin oxide, zinc oxide and sulfate, sodium hydroxide and sulfuric acid. Environmental levels of H_2S were determined by personal sampling techniques. In no case was the H_2S level greater than 1 ppm. The other potential exposures were not measured; patterns of use of those substances, however, were observed by the NIOSH industrial hygiene staff, and were deemed to be free from potential dangerous exposure.

Exposed workers were divided into three groups on the basis of their current job title: definitely high (DH), medium or moderate (M), and definitely low (DL). The classification was established before beginning the field phase of the study and was the consensus of the company and NIOSH industrial hygienists, based on a knowledge of the process and the historical sampling data. The DH group was ultimately found to have CS_2 exposure greater than 7.1 ppm; the M group exposure was between 3 and 7.1 ppm; while the DL group had less than 3 ppm exposure at the time of the NIOSH study.

C. CURRENT EXPOSURES: COMPARISON WORKERS

Prior to using workers from the nylon-polyester staple, polyester filament and nylon filament plants as a control group, possible confounding exposures in each plant were studied. Table 3-3 lists the chemical exposures in the control plants, the number of samples taken, and their results. All of the levels that could be measured are considered to be safe from known serious or confounding effects. The abbreviation (C) designates the control or comparison group.

D. CUMULATIVE EXPOSURES: CARBON DISULFIDE EXPOSED WORKERS

Area samples for CS_2 vapor had been collected and analyzed since the opening of the plant in 1948; personal sampling data for carbon disulfide have been collected since 1974. This information was microfilmed, coded, edited, and entered into the computer. Since personal and area sampling results are not directly comparable, the area sampling data were used to estimate personal exposures by assuming that a consistent ratio existed over the years between the recorded area samples and the actual personal exposure experienced by the worker. The ratio of the average area sample for a given year to the current area sampling level was determined separately for each work site. This was multiplied by the current personal sample level, as determined by NIOSH, to give an estimate of the average personal exposure level for the year and worksite. In some of the low-exposure jobs, in particular, there were gaps in the area samples so that one or more years had no data. In those cases, a linear extrapolation was made between known points. While engineering controls have changed during the lifetime of the plant, personal protective gear has not been required, so the resulting estimates should represent actual worker exposure.

Cumulative exposure for each worker was determined by multiplying the number of months the worker held each job by the estimated average personal exposure of that job in the years the worker held the job. The cumulative exposure of each job the worker held was summed to obtain the cumulative exposure index (exp. ind.). The process is represented algebraically in equations 1, 2, and 3.

1. Ratio for year and site ($R_{y,s}$) =

$$\frac{\text{average area } CS_2 \text{ vapor level for year and worksite}}{\text{current area } CS_2 \text{ vapor level for worksite}}$$

2. Estimated personal exposure for year and site ($Epe_{y,s}$) =

$$R^{*}_{y,s}(\text{current personal level}_s)$$

3. Cumulative exposure index for worker =

$$\underset{y}{\text{SUM}}\ [\underset{s}{\text{SUM}}\ [Epe^{*}_{y,s}(\text{months in worksite during year for worker})]]$$

E. CUMULATIVE EXPOSURES: COMPARISON WORKERS

The cumulative exposure index for comparison workers was estimated using the site background level of CS2, 0.2 ppm, for the workplace concentration.

F. SUMMARY

Rayon staple production requires use of carbon disulfide, which is released into the plant atmosphere at several steps during production. Historical averaged carbon disulfide exposure levels in a plant with good industrial hygeine records covering a span of three decades varied from "very small" to 15-20 parts per million, depending on the area under consideration. Current occupational exposure to CS_2 was determined by attaching a personal air sampler (with charcoal tube) to numerous workers in each of the job categories during routine 8-hour shifts over a total of 12 days at two different times (March and April, 1979). After collection, the samples were frozen and later shipped for analysis. Analysis was completed by a NIOSH-approved method and showed that CS_2 exposure varied from less than 1 to about 16 ppm, confirming the general results obtained by the company's surveillance.

Company records, including area sampling data from the startup of the plant in 1948 and personal sampling data from 1974, were used to estimate the average personal exposure level for the year and worksite. Cumulative exposure for each worker was determined by multiplying the number of months the worker held each job by the estimated average personal exposure of that job in the years the worker held the job. The cumulative exposure of each job the worker held was summed to obtain the cumulative exposure index (exp. ind.).

Figure 3-1: Steps in the Manufacture of Rayon Staple

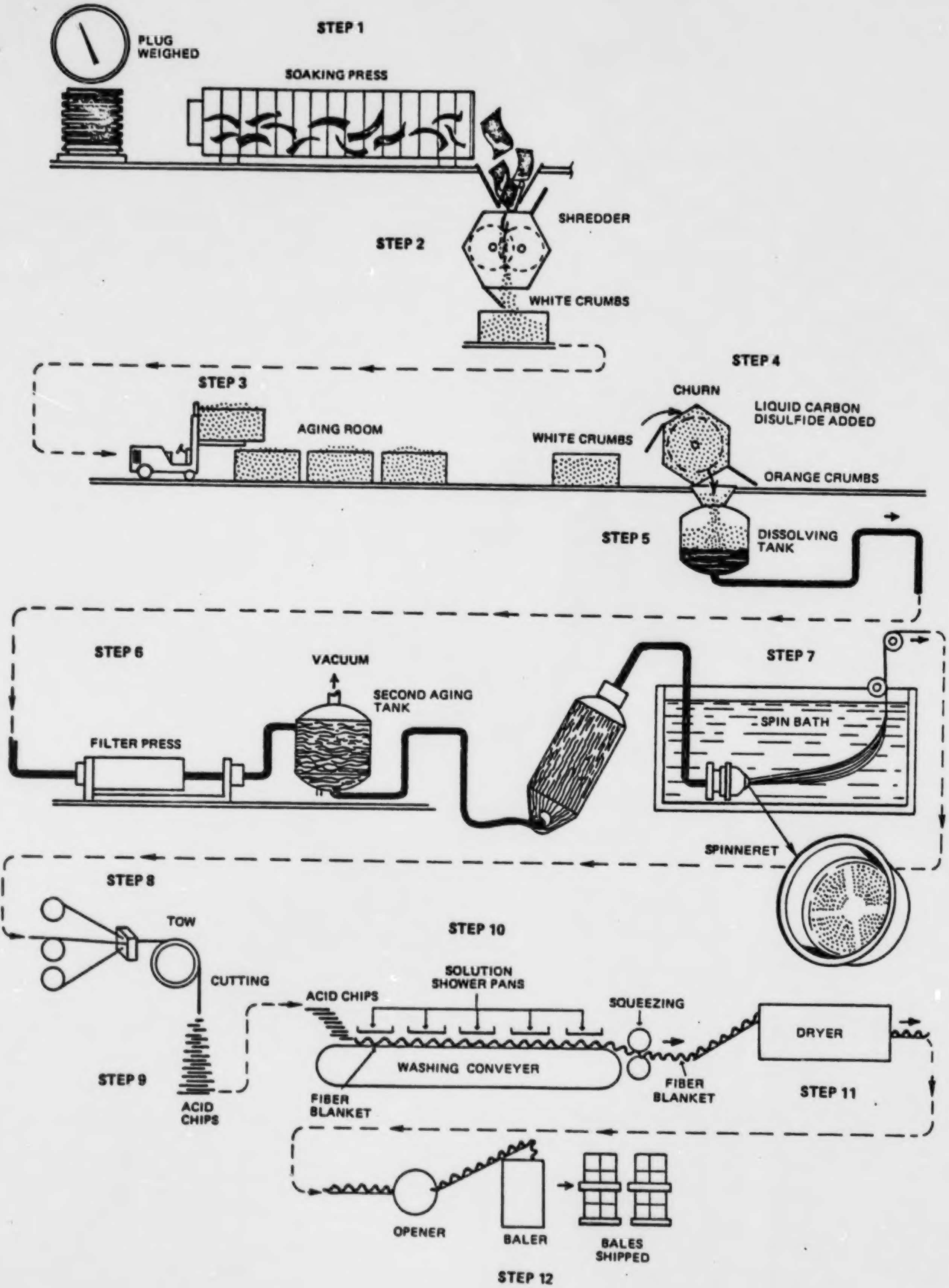
STEP 1
PLUG WEIGHED
SOAKING PRESS
SHREDDER
STEP 2
WHITE CRUMBS
STEP 4
STEP 3
CHURN
LIQUID CARBON DISULFIDE ADDED
AGING ROOM
WHITE CRUMBS
ORANGE CRUMBS
STEP 5
DISSOLVING TANK
STEP 6
VACUUM
STEP 7
SECOND AGING TANK
SPIN BATH
FILTER PRESS
SPINNERET
STEP 8
TOW
STEP 10
CUTTING
SOLUTION SHOWER PANS
ACID CHIPS
SQUEEZING
DRYER
WASHING CONVEYER
STEP 9
FIBER BLANKET
FIBER BLANKET
STEP 11
ACID CHIPS
OPENER
BALER
BALES SHIPPED
STEP 12

TABLE 3-1: Range and Average of CS_2 Samples
by Area in the Rayon Staple Plant from 1957 through 1978
(Based on Company Samples)

Step	Area	Number of Samples	Average (PPM) 1957-1978	Range
--	Waste Cutter	166	7.16	3.60 - 11.10
--	Cooling Tower	1	2.2	N/A
4	Churn Room	297	8.02	3.62 - 14.70
5	Dissolving Room	309	8.97	3.15 - 13.60
6	Spinning Machines	226	7.34	4.59 - 14.73
6	Basement Receiving Tank	157	4.99	1.99 - 9.11
6	2nd Bath Tanks	158	6.22	2.50 -230.0
6	Spinning Room	1	12.4	N/A
7	At 2nd Stretch Rollers	1281	26.09	11.35 - 29.14
7	Along hot dip	960	17.2	8.90 - 28.01
9	At the cutter	1256	19.2	8.69 - 39.48
10	Chip Conveyer	393	33.5	4.60 - 58.2
10	Correction Operator Desk	273	4.58	1.55 - 8.63

TABLE 3-2: CS_2 Exposure in the Job Categories
(Based on NIOSH Personal Exposure Measurements)

Job Description	Dept.	Process Step	N*	$\bar{x}$	S.D.	Exposure Category	
Lye Room Operator	11	1	6	0.81	0.66	DL	*
Soaking Press Operator	11	1	13	1.83	0.51	DL	*
Shredder Operator	11	2	17	5.10	3.97	DL	*
Churn Operator	11	4	30	6.70	4.08	DH	*
Dissolving Room Operator	11	5	18	4.16	1.53	M	*
Tank Cleaner	11	6	4	7.13	6.33	DH	*
Receiving and Filtration Operator	11	6	2	3.35	0.04	M	*
Spinning Tank Operator	11	6	3	3.06	0.93	M	*
Correction Operator	12	6	4	2.06	0.55	DL	*
Press Packer	12	6	13	4.69	1.14	M	*
Salt Unit Operator	12	6	4	0.16	0.16	DL	*
Crystallizer and Evaporator	12	6	4	4.24	4.54	M	*
Sand Filter	12	6	5	1.74	0.70	DL	*
Staple Spinner	12	7	55	12.64	29.09	DH	*
Tow Patroller	12	8	3	11.86	5.50	DH	*
Cutter Operator	12	9	21	9.42	6.40	DH	*
Chemical Mix Operator	12	10	3	0.58	0.35	DL	*
Dryer Operator	12	11	6	8.30	19.28	DL	*
Bail Weighing Operator	12	12	17	0.87	0.65	DL	*

*Number of person-shifts sampled
DL = Definitely Low exposure group (LT 3 ppm); M = Medium exposure group (3 to LT 7.1 ppm); DH = Definitely High exposure group (GTE 7.1 ppm)

Table 3-3: Chemical Exposures in Control Plants

Substance	N	Environmental Levels
Caprolactam	12	Single level of 10 mg 11 samples B.L.D.* (0.5mg)
Ethylene Glycol (Dowtherm)	7	Highest level 12 mg
Dimethyl terephthalate (DMT)	11	All samples B.L.D.* (0.1mg)
Methanol	6	All samples B.L.D.* (0.1mg)

* B.L.D. = Below the Level of Detection

CHAPTER 4 - STATISTICAL ANALYSIS

Of 273 male workers who were potentially available and fit the study criteria for exposed workers, 189 (69.2 percent) signed informed consent forms. For the comparison population, 422 male workers were originally asked to participate in the study; 245 (58.1 percent) signed consent forms. Because the incidence and prevalence of cardiovascular disease differ between races, and because there were only twelve non-white employees in each group, only white employees were used for the statistical analyses. Also lost to final analysis were twenty-one of the exposed workers for whom no current exposure data were obtained. 146 exposed and 233 control subjects were used for the analyses of current exposure. Fourteen additional people who did not fit admission criteria for either group were also examined and excluded from analysis.

Table 4-1: Subjects Included in Analysis

	Exposed		Unexposed	
	N	%	N	%
Available for study	273	100	422	100
Did not sign consent forms	84	30.8	177	41.9
Signed consent forms	189	69.2	245	58.1
Non-white	12		12	
White, no exposure data	21			
White, included in analysis	156		233	

Before the data were analyzed, concommitant variables and/or potentially confounding factors that might influence the outcomes were determined. When possible, adjustments for the concommitant variables and confounding factors were made in the statistical analyses. Otherwise, subjects with known confounding factors were excluded from the statistical analyses of the affected tests or measurements. Among the confounding factors considered were:

(1) report of taking medicine for high blood pressure; for high blood cholesterol, fat, or lipids; for high blood sugar or sugar in the urine;

(2) report of having diabetes, thyroid condition, or goiter;

(3) excessive alcohol consumption; and

(4) blood lead value greater than or equal to 40 mcg/dl.

The exclusion criteria were unique to each set of variables being considered.

Statistical Analysis System (SAS) was used to calculate the statistics used in this report.[1] Statistical analyses were performed using data for all participants as described in each section. Deficits in tabulations from the specified total numbers are attributable to missing values for data, which required elimination of the persons with the missing values from the particular analyses; or deliberate omissions due to the presence of confounding factors, for which control in the analysis was impractical.

Two sets of statistical analyses were performed. First, the relationships of the outcomes with current exposure were examined. Participants were classified into comparison and exposed groups. Based on the environmental data, exposed subjects were further classified into three exposure categories: definitely low exposure (DL), moderate exposure (M), definitely high exposure (DH). In this stage of statistical analysis, differences between groups were the primary bases of comparison.

Student's t-test was used to test the differences between comparison and exposed groups for variables such as age, education, cigarette packs per year smoked, thyroid function measures, fasting blood sugar, and trace minerals. The homogeneity of variances between the groups to be compared was tested. According to the homogeneity test result, the t-test for either equal or unequal variance populations was selected. For the above variables, analysis of variance was used to test the differences among the four exposure groups. The assumptions of normality and of homogeneity of the variances were checked. When violations were found, a Kruskal-Wallis test was also performed. For consistency, when the conclusions for the two analyses were the same, the F-values and p-values obtained from ANOVA were used in the summary tables and interpretations for these variables.

The variables related to serum lipids, psychological performance measures and neurologic measures were reported to be age-dependent, while blood pressure was reported to be age- and obesity-dependent. Systolic time intervals, especially QS_2 and LVET, were highly correlated with heart rate. Analysis of covariances with respective covariates such as age, age and obesity, and age and heart rate were used to test the differences between adjusted group means. The slopes of the covariates were found to be homogenous among groups, in most cases.

The group effect on correlated variables of each physiologic function was tested simultaneously using multivariate analyses of variance or covariance when appropriate. The Hotelling-Lawley trace described by Barr, et al. (1976) was used as the test criterion.[2]

The chi-square test was used to test differences between groups for categorical data such as smoking history, retinal examination results and nerve action potential responses. The GSK linear model analysis for categorical data[3] with categorizerd age group and exposure group as subsamples was used to analyze neurological symptoms.

Semen volume and sperm counts were found to be log-normally distributed, so logarithm of the observation was used in analyses. The arcsine of the square root of the proportion of abnormal sperm was used to normalize that data distribution.

In the second set of analyses, cumulative exposure to CS_2 was examined as a significant cause of variation in medical outcomes. Air sampling data for CS_2 had been collected since the startup of the plant in 1948. Personal sampling data for carbon disulfide had been collected since 1974. Based upon these data, cumulative personal exposures to CS_2 were estimated. Average exposures in ppm were determined for each job on a yearly basis. Cumulative exposure for each worker was determined by multiplying the number of months the worker held each job by the average exposure of that job in the years the worker held the job. The cumulative exposure of each job the worker held was summed to obtain the cumulative exposure index (exp. ind.).

The distribution of cumulative exposure index was skewed to the left, in part by the large number of comparison employees with only background exposure and in part by the fact that more exposed workers had low exposures than high. This is an effect often seen in concentration measurements; in order to perform some of the statistical tests, the data must be transformed to a more normal distribution. For these data, a transformation to a logarithmic scale (base 10) resulted in an acceptable approximation of normality.

For most medical measures, age is also a suspected source of variation. Therefore, age, transformed cumulative exposure index (log[exp. ind.]), and their interaction were the independent variables used in multiple regression analyses. Dependent variables used in these analyses were thyroid function, serum lipids, and fasting blood sugar. Obesity was added as an independent variable in multiple regression analysis of blood pressures, and heart rate was added for analysis of systolic time intervals. To decide if the exposure index was a significant cause of variation, the significance of the effect of log(exposure index) and its interaction with age, adjusted for the effect of age alone, age and obesity, or age and heart rate was determined. Multivariate regression analyses with the same independent variables were performed to see their effects correlated with measurements of all physiologic functions simultaneously.

Logistic multiple regression analysis was used to see if, age, log(exposure index), their interaction, and total packs of cigarettes smoked were predictors of retinal microaneurysms or hemorrhages.

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2. Morrison DF: Multivariate Statistical Methods. New York, McGraw Hill Book Co Inc, 1967.

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CHAPTER 5 - DEMOGRAPHIC COMPARABILITY OF EXPOSED AND COMPARISON GROUPS

General information including name, address, social security number, age, race, and educational level, occupational history (both at the study site and prior to employment at the study site) and smoking history was obtained from each worker by questionnaire (available on request).

A. AGE

The average age of the exposed group was 38.2 years and that of the comparison group 33.9; standard deviations were 10.11 and 9.04, respectively. This difference was statistically significant using the Student's t-test (p less than 0.01). Age was used as a covariable in the analysis of age-dependent variables. (See Table 5-1.)

B. EDUCATION

The average number of years of education for the exposed population was 10.48 years and for the comparison 11.06, with standard deviations of 2.00 and 1.76, respectively. Using the Student's t-test this difference was significant at p less than 0.01. Education was used as a covariate in the analysis of education-dependent variables.

C. OCCUPATIONAL HISTORY

Occupational history revealed that there was no significant difference in previous occupational exposure to various potentially hazardous agents between CS_2 exposed and comparison groups; analyses were not adjusted for these exposures.

D. SMOKING HISTORY

From the questionnaire results, subjects were grouped according to whether they were smokers, ex-smokers, or non-smokers (see Table 5-3). According to the X^2 test there was no significant difference in prevalence of smoking between study groups. Average pack-years similarly did not differ significantly among groups but the comparison group had a higher mean number of packs per year smoked (see Table 5-4). Analyses were generally not adjusted for smoking. An exception was made in analysis of retinal changes, since vascular effects might be especially influenced by smoking. Pack-years may provide a better measure of long-term injury from smoking than packs/year or categorical status, but pack-years correlates strongly with other time-dependent variables, including age and cumulative exposure index. Factoring smoking history into the model would tend to obscure a true relationship.

E. LENGTH OF EMPLOYMENT AND EXPOSURE INDEX

The average length of employment for the CS_2-exposed workers was 12.6 years and that of the comparison workers 8.7 years (see Table 5-5). Mean exposure index (sum of number of months in a job multiplied by the exposure level, in ppm, for that job during the time period worked) for the exposed group was 1247.9, compared to 20.8 for the comparison group, with average indices of 802.1, 1002.1, and 2077.2 for the DL, M, and DH groups, respectively. The non-zero level for the comparison group results from our finding of a low level (0.2 ppm) of CS_2 in the ambient air in plants adjacent to the rayon facility. The cumulative exposure index was not normally distributed through the workforce. We employed a logarithmic transformation and used log(exposure index) in analyses throughout the report (see Table 5-6).

Since these variables would be expected to correlate highly with one another, Pearson's product moment correlations were calculated between age, employment length, exposure index, and log(exposure index) (see Table 5-6). Age could have an effect on many of the physiologic variables measured, as could exposure to CS_2. Because of the high correlation, use of employment length as an independent variable would tend to mask effects of age and exposure; employment length was therefore not used as a variable in analyses done by the Division of Surveillance, Hazards Evaluations, and Field Studies. The neurological tests were analyzed by the Division of Biomedical and Behavioral Sciences, where employment length was used as an independent variable. The high degree of correlation between age and exposure index (0.40) suggests that their interaction should be considered in analysis of data.

F. LIVER ENZYME TESTS

Gamma-glutamyl transpeptidase (GGTP) was measured on all blood samples drawn, in part as a screening test for unreported alcoholism. For those samples with an abnormal GGTP, serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were measured. Elevation of these enzymes occurs with inflammatory diseases of the liver. The results are displayed in Table 5-7, which shows no significant differences among the groups.

G. DISCUSSION

The greatest single difference between the comparison and exposed populations is in average age. Biological effects of age were handled by adjusting age-dependent variables for age or by using age as a covariable in the analyses. The possibility of non-biological cohort effects is always a possibility in epidemiologic studies, and is suggested here by the lower mean years of education and the lower rates of smoking among the exposed groups and by unexplained differences in blood lead level (see chapter 6). While there is no clear reason to believe that cohort effects account for a net bias toward either positive or negative findings, these differences in groups should be borne in mind.

H. SUMMARY

Statistically significant differences between exposed and comparison groups were found in age, educational level, length of employment, and exposure index (sum of number of months in a job multiplied by the exposure level, in ppm, for that job during the time period worked). Mean exposure index (sum of number of months in a job multiplied by the exposure level, in ppm, for that job during the time period worked) for the exposed group was 1247.9, compared to 20.8 for the comparison group, with average indices of 802.1, 1002.1, and 2077.2 for the DL, M, and DH groups, respectively. The number of packs smoked per year by the comparison group was slightly greater than that for the exposed group. There were no significant differences between groups in history of prior occupational exposure to CS_2 or in liver enzyme levels. Age, length of employment, and exposure index were highly correlated with each other. A logarithmic transformation of the exposure index was used to render the distribution more nearly normal.

Table 5-1: Age (Years)

	Number	Mean	S.D.	Median	Minimum Value	Maximum Value
Comparison Participants:						
C	233	33.9	9.02	32.0	19.6	61.6
Exposed Participants:						
DL	46	37.3*	10.9	35.0	22.2	61.5
M	65	38.4*	9.4	36.8	17.5	63.1
DH	44	39.0*	10.4	38.6	22.7	58.2
All Exposed Participants Combined:						
EXP	155	38.3*	10.1	36.3	17.5	63.1

*Significantly different from Comparison Participants, but not significantly different from each other (Duncans multiple range test, p less than 0.05).

Table 5-2: Length of Employment (Years)

	Number	Mean	S.D.	Median	Minimum Value	Maximum Value
Comparison Participants:						
C	233	8.7	4.9	7.9	0.9	28.8
Exposed Participants:						
DL	46	11.2*	7.3	9.4	1.0	30.8
M	65	12.9*	6.2	11.2	1.0	30.7
DH	44	13.7*	7.2	11.5	3.1	31.0
All Exposed Participants Combined:						
EXP	155	12.6*	6.9	11.0	1.0	31.0

*Significantly different from Comparison Participants, but not significantly different from each other (Duncans multiple range test, p less than 0.05).

C = control group; DL = low exposure group (less than 3ppm); M = medium exposure group (3 to 7.1 ppm); DH = high exposure group (greater than 7.1 ppm)

Table 5-3: Smoking Histories of Exposed and Comparison Workers: Present Smokers, Ex-Smokers, and Non-Smokers

	Comparison		DL		M		DH		All Exposed	
	N	%	N	%	N	%	N	%	N	%
Smokers	130	56 %	30	65 %	34	53 %	20	45 %	84	55 %
Ex-Smokers	63	27 %	9	20 %	17	27 %	11	25 %	37	24 %
Non-Smokers	39	17 %	7	15 %	13	20 %	13	30 %	33	21 %
Totals	232	100 %	46	100 %	63	100 %	44	100 %	154	100 %
Chi-Square & (P) value			6.15 (0.41)						1.45 (0.50)	

Table 5-4: Smoking Histories of Exposed and Comparison Workers: Packs per Year and Pack-Years

		Comparison	DL	M	DH	All Exposed
Packs/year	N	226	46	63	43	152
	Mean	359	329	323	268	310
	S.D.	236	215	227	218	221
F* & (P) value			2.06 (0.10)			2.05 (0.04)
Pack-Years	N	225	45	63	42	150
	Mean	6178	5675	6425	6178	6122
	S.D.	5888	4566	5796	7213	5849
F* & (P) value			.14 (0.93)			.09 (0.93)

* t-test is used for the comparison of Comparison vs. All Exposed.

Table 5-5: log(Exposure Index)

	Number	Mean	S.D.	Median	Minimum Value	Maximum Value
Comparison Participants:						
C	233	1.22	0.32	1.28	0.32	1.84
Exposed Participants:						
DL	46	2.57*	0.64	2.75	0.92	3.63
M	65	2.89*	0.34	2.96	1.82	3.55
DH	44	3.16**	0.42	3.18	1.70	3.85
All Exposed Participants Combined:						
EXP	155	2.87**	0.52	2.96	0.92	3.85

* Significantly different from Comparison Participants and from each other. (Duncans multiple range test, p less than 0.05).

** Significantly different from Comparison Participants. (p less than 0.05).

Table 5-6: Pearson's Product Moment Correlations
Age, Employment Length, Exposure Index, and log(Exposure Index)
N=388

	Age	Employment Length	Exposure Index	log (Exposure Index)
Age	1.0			
Employment Length	0.65	1.0		
Exposure Index	0.40	0.52	1.0	
log(Exposure Index)	0.39	0.60	0.76	1.0

All p less than 0.0001

Table 5-7: Hepatic Enzyme Levels* of Exposed and Comparison Workers

		Comparison	DL	M	DH	All Exposed
GGTP (mU/ml)	N	213	43	59	43	145
	Mean	26.1	18.4	22.0	22.6	21.1
	S.D.	24.8	7.3	18.7	14.3	14.8
Kruckal-Wallis X^2 & (P) value			5.64 (0.13)			3.55 (0.06)
SGOT (U/1)	N	32	1	7	4	12
	Mean	26.4	27.0	27.9	21.0	25.5
	S.D.	11.6	--	12.9	49.9	10.4
F* & (P) value			0.33 (0.80)			0.25 (0.81)
SGPT (U/1)	N	32	1	7	4	12
	Mean	44.2	23.0	54.6	36.0	45.8
	S.D.	25.8	--	37.2	14.3	30.7
F* & (P) value			0.66 (0.58)			-0.17 (0.86)

Gamma-Glutamyl Transpeptidase (GGTP), Serum Glutamic-Oxalacetic Transaminase (SGOT), and Serum Glutamic-Pyruvic Transaminase (SGPT)

CHAPTER 6 - CARDIOVASCULAR EFFECTS

A. ANGINA AND MYOCARDIAL INFARCTION

1. Literature Review

In 1938, the Pennsylvania Department of Labor and Industry described for the first time a possible relation between heart disease and carbon disulfide exposure. The report said that one third of 110 viscose rayon workers had subjective complaints of shortness of breath, chest pain, faintness, and poor exercise tolerance.[26] No objective information concerning the incidence or prevalence of angina or myocardial infarction was obtained.

In the late 1960's, Tiller[27] performed a retrospective cohort mortality study of three viscose rayon factories in the same British county, obtaining evidence that angina and myocardial infarction were caused by CS_2. Of 397 rayon process workers (viscose makers and spinners) who died between 1933 and 1962, 42 percent died of coronary heart disease (94 observed versus 42 expected, p less than 0.001).

Hernberg et al.[12] studied coronary vascular disease morbidity in 343 CS_2-exposed and 343 comparison Finnish workers (matched by age) using the cardiovascular disease questionnaire approved by the World Health Organization in 1963. The questionnaire is designed to classify chest symptoms according to severity and probability of coronary etiology. A statistically significant (p between 0.010 and 0.025) increase in a history for angina was found in the exposed group compared to the comparison. The same questionnaire showed a slight increase in the prevalence of a history of myocardial infarction in the comparison group, but the difference was not statistically significant.

In a 5 year follow-up of the same cohorts, Tolonen et al.[29] again found a statistically significant increase in history of angina in the exposed group (p less than 0.0002). Additionally, during the five year period, 14 exposed cohort workers and 3 comparison workers died from coronary heart disease (p less than 0.007) and 11 exposed and 4 comparison workers had non-fatal infarctions.

By contrast, Vertin[31] studied the Dutch viscose rayon industry using a standardized coronary risk profile which included subjective data dealing with chest pain and other factors and found no statistical difference between a group exposed to less than 20 ppm CS_2 and a non-exposed group. The analyses did not control for age, which differed significantly among the groups.

In view of the substantial body of evidence that occupational exposure to CS2 could be injurious to the cardiovascular system, this study was designed to obtain additional evidence about the dose-response relationships in the exposure range below 20 ppm and to correlate a number of independent variables and results in a single population.

2. Methods

The Rose Questionnaire[21] (Appendix II), which is similar to one adopted by the World Health Organization, was used to determine prevalence of angina pectoris and myocardial infarction. The Rose Questionnaire was administered to each employee individually by a trained interviewer, who was unaware of the subject's exposure status and who was instructed to give the questionnaire without resorting to probing questions.

3. Results

a. By Current Exposure

Table 6-1 shows that one worker from the exposed cohort could be diagnosed as having angina by the Rose criteria. The prevalence of angina was therefore 0.64 percent in that group. No workers in the comparison group had angina. A total of five workers, three from the exposed group (prevalence 1.92 percent) and 2 from the comparison group (prevalence 0.86 percent) were shown to have had a myocardial infarction by the questionnaire (overall prevalence 1.3 percent). By taking all of the subjects diagnosed as having either angina or myocardial infarction, a prevalence of 2.58 percent was obtained for both forms of coronary heart disease in the exposed group. Results are not statistically significant; for combined angina and myocardial infarction, the exposed vs. control comparison had an odds ratio of 3.1 but Fisher's exact test showed $p = .18$ (one tail).

b. By Cumulative Exposure

In view of the small numbers of cardiovascular abnormalities found, no statistical analyses of prevalence by cumulative exposure index were performed.

4. Discussion

From the 1960-62 National Center for Health Statistics (NCHS) National Health Survey,[11] the prevalence of "definite" coronary heart disease (both myocardial infarction and angina) was 2.8 percent in the adult U.S. population. Their diagnoses were made by medical history and electrocardiograms. The population studied consisted of adult men and women and was not composed entirely of working people.

Hernberg, using the Rose Questionnaire in his previously cited study of Finnish viscose rayon workers, found 1.2 percent "verified" M.I.'s in exposed workers and 2.4 percent in the comparison group.[12] He also found "typical" angina in 7.1 percent of the exposed workers and in 5.6 percent of the comparison group. Hernberg used the Rose Questionnaire to further subdivide angina into typical, probable, and possible cases.

This maneuver brought the total percent of cases of angina to 16.8 percent in the exposed group and 10.6 percent in the comparison group.

At first glance, it appears that the NIOSH study cohort has a better health record than either the NCHS or the Finnish cohort. That is a deceiving picture for several reasons. The so-called healthy worker effect favors low prevalence in the NIOSH worker cohort compared to the NCHS general population cohort; the fact that both NCHS and Finnish studies used physicians as interviewers may have increased the rate of discovery of real cases, resulting in a higher perceived prevalence than in the NIOSH study (NIOSH interviewers were instructed to give the Rose Questionnaire only as it was written; no probing questions were permitted); and there is a significantly higher rate of coronary disease in Finland than in the United States. Our interviewing technique might have acted to decrease case finding, because questions obviously misunderstood by the subjects were not followed by clarifying questions from the interviewers. Whether this strict tactic was used by Hernberg et al. is not known. Hernberg's use of the WHO Questionnaire to qualify responses (i.e. typical, probable, possible) improved case finding as compared to the present study, in which only "typical" cases were counted. Finally, the exposure levels in the Finnish plant appear to have been slightly greater than in this plant, so the difference may actually reflect an effect of exposure.

The above differences in case definition of coronary heart disease make detailed comparison of these studies impossible. Certainly, some set pattern of questions needs to be followed for meaningful, unbiased field studies. Geoffrey Rose[21] developed a questionnaire for use in field surveys that would "identify those characteristics of angina pectoris, cardiac infarction ... which most effectively distinguish these conditions from other causes of chest ... pain". His hope was that such a questionnaire could serve as a standard for international comparison.

In our review of each subject's Rose questionnaire after completion of the study, it was obvious that mistakes were made by both subjects and interviewers. The occurrence of these errors raises doubt about the validity of the Rose questionnaire as it is presently used; in the future, the Rose questionnaire should be modified by rephrasing questions to improve understanding, by adding standard explanations, or by having a physician or paramedical person familiar with heart disease administer the questionnaire, adding interpretation as needed. Revalidation of the new instrument would then be required.

In the planning phase of this study, we estimated that we would have an 80% chance of detecting a prevalence of 5.5% among the exposed population providing: 1) the prevalence in the comparison population was of 2.5% and 2) at least 300 workers in each group could be examined. Neither prerequisite was met. Calculations of statistical power show that with a comparison population incidence of 0.86%, we would need 1285 subjects, equally divided between exposed and controls, to have even a 50% chance of detecting a doubling in the frequency of angina.

In summary the results of this section of the study are inconclusive due in part to an inadequate determination of cases of angina and myocardial infarction and in part to the small size of our sample.

B. BLOOD PRESSURE

1. Literature Review

Numerous studies have suggested a relationship between CS_2 exposure in rayon viscose workers and hypertension. Hernberg's measurement of systolic and diastolic blood pressures revealed statistically significant elevations (4-6 mm Hg) in the exposed cohort.[12] A follow-up of this same group by Tolonen et al. 5 years later again showed elevated systolic and diastolic blood pressures in exposed workers.[29] CS_2 exposures ranged from 10-40 ppm in both studies.

In 1976, Tolonen et al.[28] performed a cooperative Finnish-Japanese study looking again at blood pressures among other variables. The exposed Finnish workers had significantly higher systolic and diastolic blood pressures than their controls. There were no significant differences in Japanese exposed vs. comparison workers despite the fact that levels of exposure to CS_2 in the Finnish and Japanese groups were similar (5-60 ppm).

Lieben et al.[16] compared blood pressure measurements in viscose rayon workers and a group of acetate workers. He found the average blood pressure of rayon workers to be 140/87 and the average for acetate workers to be 135/83. These differences were not statistically significant. Moreover, acetate can alter cardiovascular performance and lower blood pressure.

2. Methods

Two blood pressure determinations were taken in each worker after the worker had been in a supine position and an electrocardiogram had been recorded (average rest = 10 minutes). A Random Zero Sphygmomanometer was used for the determinations. This device is fitted with a zero shifting device that precludes reading the actual blood pressure until after the determination is made (thus eliminating the digit preference bias)[33]. For each determination, Phases I (the point at which sounds first appear), IV (the point at which sounds change abruptly), and V (the point at which sounds disappear) of the blood pressure were recorded. For statistical analysis the average of the two determinations was used.

Workers who reported taking high blood pressure medication, "water pills," diuretics, ACTH, or corticosteroids were excluded from analysis.

3. Results

a. By Current Exposure

Table 6-2 shows the blood pressures by group; the differences which existed between all three phases of blood pressure measured became smaller after age and obesity adjustments. In this analysis the systolic (Phase I) reading of all exposed workers was significantly higher than the comparison group at $p = 0.05$. No significant differences were found in analysis of the comparison vs. DL, M, and DH groups, although the means of the three groups were uniformly higher than the comparison group's mean and increased with exposure level. Somewhat similar trends were seen in Diastolic IV and V measurements, although the means did not differ significantly at the $p = .05$ level.

b. By Cumulative Exposure

Figures 6-1 through 6-3 are scattergraphs of systolic blood pressure and diastolic IV and V blood pressures vs. the logarithm of the cumulative exposure index (log(exp. ind.)). Table 6-3 shows the analysis of systolic (Phase I) and diastolic (Phases IV and V) blood pressures by cumulative exposure. Each Phase was regressed on age, log(exp. ind.), the interaction of age and log(exp. ind.), and obesity. Obesity index is the quotient of present body weight in Kg and ideal body weight estimated by:

$$\text{Ideal body weight}_{(kg)} = 0.9(\text{Height}_{(cm)} -100)$$

The exposure index (exp. ind.) is the product of the historical levels of CS_2 in each department by year and the number of months the employee spent in that job during the year for which the level was applicable. The R^2 due to the log(exp. ind.) and its interaction with age measures the amount of variation of the outcome (BP measurements) explained jointly by these two variables. The results of multivariate analysis of covariance (MANCOVA) are presented in the cell at the bottom of the table.

The test of significance (F value), for whether the increased variance accounted for by the regression with the log(exp. ind.) and interaction terms is given as well.

Log(exp. ind.) and its interaction with age are significantly correlated with systolic blood pressure but jointly explain only 0.033 of the variation of systolic blood pressure. Log(exp. ind.) and its interaction do not jointly explain a significant amount of variation in diastolic blood pressure (Phase IV or V). When the variables are considered as a group, using MANCOVA, the associations of the set of three blood pressure readings with log(exp. ind.) and with log(exp. ind.) and its interaction with age are significant.

4. Discussion

Table 6-2 shows that, after age and obesity adjustments are made, all mean blood pressure values save the Diastolic V reading of "DL" are numerically higher in those subjects who have been exposed to CS_2 than in controls. Except for "DL" Diastolic V, there is also a trend toward higher blood pressures when the amount of exposure is greatest. The actual numerical differences are not great (up to 2.65 mm Hg), and are not as impressive as those in Hernberg's 1970 study[12], which found statistically significant increases of 4-6 mm Hg in the 3 phases of blood pressure, nor are they as impressive as the differences found in Tolonen's 1975 study[29], in which statistically significant differences of 3.5-8.0 mm Hg were found. The differences in the Finnish and American experience may be related to a lower CS_2 exposure level in this study (less than 15 ppm) as compared to the Finnish study (10-40 ppm) and to population differences suggested by the generally higher incidence of coronary heart disease in Finland than in the U.S.

The analysis by cumulative exposure shows a statistically significant effect of log(exp. ind.) on the set of blood pressure readings, with most of the effect manifest in systolic blood pressure.

C. THE ELECTROCARDIOGRAM

1. Literature Review

The electrocardiographic (ECG) findings of ischemic heart disease are well documented. In epidemiologic studies, the sensitivity of the ECG to detect coronary heart disease can be increased by stress testing.

In Hernberg's 1970 Finnish viscose rayon study[12], resting 12-lead ECG's were recorded, followed by standardized bicycle ergometer stress with ECG being recorded during and after the exercise. The ECG findings were classified according to the Minnesota Code. Twenty comparisons were used involving different exposure parameters and different sections of the Minnesota Code; none of the tests showed statistical differences between exposed and comparison groups.

Tolonen's 1975 five year follow-up of the Hernberg study[29] used exactly the same techniques for ECG measurement as Hernberg's. Again no significant differences between exposed and comparison groups were found.

The 1976 collaborative Japanese-Finnish study by Tolonen et al.[28] used similar ECG techniques and coding. The results showed no remarkable excess prevalence of changes reflecting coronary heart disease on ECG's in either Finnish or Japanese groups.

Lieben[16], in his 1974 study, obtained electrocardiograms from exposed and comparison groups. No description of the ECG techniques was included in the report. ECG strips were read blindly and coded by a 10 point system. No significant differences between study groups were found.

Vertin[31] in his study of Dutch viscose rayon industry used the Minnesota Code to evaluate study groups. He found no significant difference in the indicators of coronary insufficiency.

2. Methods

Resting ECG's were recorded by a Hewlett-Packard 3-channel electrocardiographic machine while the subject was in a supine position. The ECG strips were mounted and stored for later shipment to the readers.

Each ECG was read separately by two cardiologists and recorded using the Minnesota Code[2] (See Appendix III). After all ECG's were coded, the cardiologists reviewed those for which there was a discrepancy and then came to a consensus. The consensus reading was used for subsequent statistical analysis.

3. Results

a. For Current Exposure

Although the entire Minnesota Code was used to grade the ECG's, only those categories thought to be important in coronary heart disease are reported in Table 6-4. The important categories are Q and QS pattern, S-T junction and segment abnormalities, and T-wave abnormalities. There were too few abnormalities to warrant statistical analysis.

b. For Cumulative Exposure

In view of the small number of abnormalities, analyses by cumulative exposure were not conducted.

4. Discussion

During the design of this study, it was realized that a maximal stress electrocardiogram is considered superior to a resting ECG for the diagnosis of coronary artery disease. The experience of Hernberg[12] and Tolonen[28,29] suggested that "... an exercise ECG provides an imperfect means for detecting potential excess CS_2-induced CHD morbidity". Given the poor results of stress testing in earlier CS_2 studies, plus the constraints of time and location in the present study, the stress ECG was abandoned in favor of a resting ECG. Some specificity may thereby have been sacrificed.

This study and the preceeding studies mentioned in the literature reflect the weakness of the ECG in diagnosing coronary artery disease. The results of these analyses can not be taken as proof that CS_2 does not cause coronary artery disease. The ECG is only one of many tools useful in making a diagnosis of coronary artery disease.

D. CARDIAC RATE AND CONTRACTILITY

1. Literature Review

Previously published studies have suggested an association between carbon disulfide exposure and coronary heart disease.[12,28,29] Other surveys indicate possible associations between CS_2 exposure and a pre-diabetic state.[9,25]

Weissler[32] and Ahmed et al.[1] indicate that either coronary disease or diabetes can cause an increase in the systolic time interval (STI) which is measured from simultaneous determination of ECG, carotid pulse wave, and phonocardiogram. Franco and Malamani[8] determined the STI's of 18 viscose rayon workers (exposed to 20 ppm) and 22 comparison workers (metal arc welders). They found a shortened left ventricular ejection time (LVET), prolonged pre-systolic ejection period (PEP) and prolonged isovolumetric contraction time (ICT). These values produced a PEP/LVET ratio similar in both exposed and controls but an ICT/LVET ratio of significantly greater value in the exposed group. Although PEP/LVET is usually used to determine an altered STI, both ratios have been used. In this instance Franco and Malamani suggested that the increased ICT/LVET ratio was due to an alteration in myocardial contractility caused by CS_2 exposure.

2. Methods

The systolic time interval was determined by simultaneously recording a standard ECG lead, phonocardiogram, and carotid pulse wave. The equipment used included a Hewlett-Packard 4588D Optical Recorder with Rapid Developer, H-P 8805 Pressure Modifier with P23 DG Stratham Strain Gauge, an H-P 8811-D ECG Amplifier, and an H-P 8813-A Heart Sound Amplifier with H-P 21050-A Heart Sound Microphone. All STI's were recorded by the same researcher (M.B.). At least 10 heart cycles were recorded; in a random number of subjects (approximately every 10th recorded STI) 20 heart cycles were recorded and split in half for duplicate readings. After recordings were made, the recording sheets were mounted and stored for future determinations of STI. Heart rate was determined by palpation of a peripheral artery twice during the physical examination.

The QS_2, LVET, and PEP were determined from each of the ten complexes (Figure 2). The mean and standard deviation of each ten intervals (i.e. 10 QS_2, 10 LVET, 10 PEP) were determined and adjusted by multivariate analysis of variance according to the subjects age and heart rate. Every tenth recording was recalculated independently by an STI technician.

Subjects with a murmur consistent with aortic stenosis were excluded from this procedure because of excessive risk of complications. Also results are not reported for individuals with:

a. History of taking digitalis or antiarrhymic drugs.

b. ECG showing left axis deviation (beyond -30°), P-R interval greater than 0.21 sec, or left bundle branch block.

c. Tracing either not readable or carotid upstroke too flat for accurate reading.

The literature suggests that these factors may result in erroneous measurements of STI.[15,32]

3. Results

a. For Current Exposure

Heart rate was slightly faster for exposed than for comparison groups, with a steady increase as exposure increased; the differences were statistically significant. The means of all the standard STI measurements, adjusted for age and heart rate, are given in Table 6-5. In all cases, the difference between exposed and comparison groups is insignificant.

b. For Cumulative Exposure

Figures 6-4 through 6-7 are scattergraphs of QS_2, LVET, PEP, and PEP/LVET vs. log(exp. ind.). QS_2, LVET, PEP, and PEP/LVET were each regressed on age, log(exposure index), and heart rate, and the interactions of age with log(exp. ind.) and heart rate with log(exp. ind.), where the exposure index (exp. ind.) is the product of the historical levels of CS_2 in each department by year and the number of months the employee spent in that job during the year for which the level was applicable. Results are given in Table 6-6. The incremental R^2 (due to log(exp. ind.) and its interaction with age) was not statistically significant for any variable. Multivariate analysis of covariance showed no significant association between exposure index or any of its interaction terms and any STI measurement. Figure 6-8 is a scattergraph of heart rate vs. log(exp. ind.). As seen in Table 6-7, there was a statistically significant correlation between heart rate and the interaction of age with log(exp. ind.), but the clinical significance of that finding is obscure, at best. The full model, considering effect of hemoglobin on heart rate as well as age and exposure, explained about 6.4% of the variance.

4. Discussion

Since the usual tools available to field researchers for the diagnosis of heart disease (i.e. ECG, history) usually detect only clinical cases of heart disease, the systolic time interval was used as a possible preclinical detector of CHD (i.e. diminished left ventricular performance).[1,8,32] Even though the STI may detect preclinical heart disease, some patients with clinical angina and even history of myocardial infarction will have a normal STI.[32]

This study has found no significant shortening of the left ventricular ejection time (LVET), lengthening of the presystolic ejection period (PEP) or increase in the PEP/LVET ratio as Franco and Malamani found in a comparable study of CS_2 exposed workers.[8] While these data do not rule out the possibility that CS_2 causes heart disease, they do suggest that left ventricular performance is not demonstrably affected by exposure to CS_2 at the exposure levels encountered in this study group.

E. LIPID METABOLISM

1. Literature Review

For some time, altered serum lipid levels have been considered as risk factors for coronary heart disease. Increased risk of coronary heart disease was associated with increases in total serum cholesterol and triglyceride. As better laboratory techniques have become available, it has been found that the risk is particularly associated with elevation in low density lipoprotein (LDL) levels, with diminution in high density lipoprotein (HDL) levels, and accordingly with elevation in the LDL/HDL (low density lipoprotein/high density lipoprotein) ratio.

Numerous studies of serum lipid levels have been performed involving workers exposed to carbon disulfide. There are some reports that serum cholesterol is elevated in workers exposed to CS_2[10,17]. Other studies have shown increased beta lipoproteins (low density lipoproteins) in exposed subjects[20,22]. Harashima[10] found that amount of exposure to CS_2 correlates with serum cholesterol. In his study, workers chronically exposed to 15-65 ppm of CS_2 had higher serum cholesterol levels than those exposed to 5-19 ppm and an unexposed group of workers. This same group of researchers performed another study[30] in which they showed that in Japan during 1951-1953 workers exposed to levels of 40-50 ppm of CS_2 with peaks of 300 ppm had increased serum cholesterol levels compared with unexposed workers.

Serum cholesterol levels obtained by Harashima in 1965 from the same exposed workers (only 5-15 ppm exposure) and non-exposed controls, showed no difference.

2. Methods

Serum cholesterol, triglycerides, high density lipoprotein, and low density lipoprotein were determined on serum obtained from blood drawn from fasting subjects. Workers who reported taking medicine for high cholesterol, high blood fat, or high lipids in the last month were excluded from analysis, as were workers with a history of thyroid disease.

3. Results

a. From Current Exposure

Table 6-8 shows means and age adjusted means of serum cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and LDL/HDL ratios. No significant differences between comparison and exposed groups is present.

b. By Cumulative Exposure

Figures 6-9 through 6-13 are scattergraphs of cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, and ratio of low to high-density lipoproteins vs. log(exp. ind.). Table 6-9 shows the analysis of blood lipids by cumulative exposure. Each lipid test and the ratio of low density to high density lipoproteins was regressed on age, log(exp. ind.), and the interaction of age and log(exp. ind.). The R^2 due to log(exp. ind.) and its interaction with age measures the amount of variation of the outcome explained jointly by these two variables. The test of significance (F value) of the incremental R^2 due to log(exp. ind.) and its interaction with age is given as well.

Log(exp. ind.) and its interaction with age are significantly correlated with cholesterol, triglycerides, low density lipoproteins (LDL), and LDL/HDL ratio. Log(exp. ind.) and its interaction with age jointly explain 0.027 of the variation of cholesterol, 0.028 of the variation of triglycerides, 0.17 of the variation of low density lipoproteins, and 0.017 of the variation of LDL/HDL ratio.

Log(exp. ind.) and its interaction do not jointly explain a significant amount of variation of high density lipoproteins (HDL).

4. Discussion

Some earlier work suggested that serum lipid components may be altered with exposure to CS_2. Clinically useful prognostic tests of lipid metabolism were performed to see whether CS_2 exposure correlates with abnormal lipid patterns.

Analysis by acute exposure shows no statistically significant difference between exposed and comparison groups in any of the variables studied. A smooth increase in mean LDL from comparison, to DL exposure, to M exposure, to DH exposure which may be seen in the unadjusted data is disturbed when the data are adjusted for age, but the trend still appears. Clinically a higher LDL indicates increased risk of CHD. The differences noted in the analysis of cumulative effect are statistically significant, but explain a very small fraction of the variance observed.

This information does tend to confirm the findings of other studies which have shown abnormalities in lipid metabolism but suggests that at or below the level of 20 ppm, CS_2 has a minimal effect on lipid metabolism.

F. TRACE MINERAL EXCRETION

1. Literature Review

Carbon disulfide is metabolized to dithiocarbamates and thiazolidones, which are considered capable of chelating polyvalent metals. The chelated complexes may then be lost through urinary excretion, depleting body stores of essential minerals. Zinc and copper deficiencies in particular have been implicated in the pathophysiology of CS_2 poisoning[3,4,5,6,19,23,24].

Massoud et al.[18] report impressive declines in serum copper and ceruloplasm in rats treated with CS_2. El-Dessoukey et al.[7] report similar changes in serum zinc, iron, calcium, and magnesium, with increases in potassium and a slight decrease in sodium.

Hernberg et al.[13] reported urine zinc excretion decreased slightly after 10 workers began CS_2 exposure in the spinning room of a rayon plant. They did not consider the differences significant. Erythrocyte magnesium and zinc were slightly lower in Finnish workers exposed to CS_2, while plasma magnesium was slightly higher.[14] The differences were statistically significant, although the clinical significance is not obvious. A smaller group of workers exposed in a Norwegian plant showed no significant differences in any of the measurements.

Blood lead changes are not associated with carbon disulfide exposure, but elevated body lead burden could be a confounding factor for some of the neurological and neuropsychiatric tests if not distributed evenly among exposed and comparison subjects. A measurement of blood lead was therefore included in the analyses done on blood samples.

2. Methods

Blood lead, serum zinc, and serum copper were determined from the blood samples collected. Urine zinc concentration was determined from samples collected from a small number of controls and medium and high exposure subjects.

3. Results

Descriptive statistics are given in Table 6-10. There are no statistically or clinically significant differences between groups in serum zinc, serum copper or urine zinc. There was no significant difference between exposed and comparison groups in blood lead. When analyzed by level of exposure, there was a regular increase in lead level in the groups more highly exposed to CS_2, with the definitely high group showing about twice the blood lead level of the comparison group. The highest level was 17.88, which is within the normal range given by most laboratories, so the clinical significance of this observation is minimal.

4. Discussion

Exposure to carbon disulfide in the range below 20 ppm has no effect on zinc or copper metabolism demonstrable by the tests used here. Differences in serum protein, while statistically significant, do not appear clinically important. Blood lead levels are surprising; the means reported are all within a range considered normal, but individual observations in each group lie well outside the normal range and represent excess absorption. While lead is used in the plant, it seems likely that the exposures occur outside the work environment. The importance to this study lies in the possible confounding effect of lead on neurological and psychological measurements.

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H. SUMMARY

A cross-sectional medical and industrial hygeine survey of workers exposed to CS_2 in a rayon staple plant and of comparison workers in other plants at the same site examined history of coronary disease, blood pressure, electrocardiograms, systolic time intervals, blood lipids, serum zinc and copper, blood lead, and urine zinc. Analyses were conducted for both current exposure levels and cumulative exposure index.

The Rose Questionnaire[21] (Appendix II), which is similar to one adopted by the World Health Organization, was used to determine prevalence of angina pectoris and myocardial infarction; the prevalences of angina and myocardial infarction were too small to permit conclusions.

Differences of up to 3.1 to 3.8mm Hg, which decrease to a range of 2.1 to 2.7mm Hg after age and obesity adjustments, exist between exposed and control groups for all three phases of blood pressure measured; the systolic (Phase I) reading is significantly higher for exposed than for comparison workers. The differences are correlated with exposure level, rising to a maximum of 4.9mm Hg (systolic pressure, adjusted for age and obesity) between the DH and control groups.

Log(exp. ind.) and its interaction with age are significantly correlated with systolic blood pressure but jointly explain only 0.033 of the variation of systolic blood pressure; they do not jointly explain a significant amount of variation of diastolic blood pressure (Phase IV or V).

There were too few ECG abnormalities to draw conclusions. No significant group differences in systolic time intervals were found. A statistically significant correlation between heart rate and the three variables log(exp. ind.), interaction of age with log(exp. ind.), and interaction of hemoglobin with log(exp. ind.) explained about 5% of the variation, but the clinical significance of that finding is obscure, at best.

No significant differences between comparison and exposed groups is present for serum cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and LDL/HDL ratios. Log(exp. ind.) and its interaction with age are significantly correlated with cholesterol, triglycerides, low density lipoproteins (LDL), and LDL/HDL ratio but jointly explain only 0.027 of the variation of cholesterol, 0.028 of the variation of triglycerides, 0.17 of the variation of low density lipoproteins, and 0.017 of the variation of LDL/HDL ratio; they do not jointly explain a significant amount of variation of high density lipoproteins (HDL).

There are no statistically or clinically significant differences between groups in serum zinc, serum copper or urine zinc. There was no significant difference between exposed and comparison groups in blood lead but when analyzed by level of exposure, there was a regular increase in lead level in the groups more highly exposed to CS_2.

Table 6-1: Number of Participants with History of Angina or Myocardial Infarction By Current Exposure Category (Control versus DL, M, DH, and All Exposed)

	Angina		Myocardial Infarction		
	N	Percent	N	Percent	Number
Control	0	0.00	2	0.86	233
DL	1	2.13	0	0.00	47
M	0	0.00	2	3.08	65
DH	0	0.00	1	2.27	44
All Exposed	1	0.64	3	1.92	156

Table 6-2: Average Blood Pressure By Current Exposure Category (Control versus DL, M, DH, and All Exposed)

		Control N = 221	DL N = 43	M N = 57	DH N = 43	All Exposed N = 143	Difference Exposed -Control
Systolic (mm Hg)	$\bar{x}$	126.68	129.14	130.20	132.10	130.45	3.77
	S.D.	11.55	14.55	14.41	15.50	14.13	
	$\bar{x}$ (age)	127.11	128.52	129.06	132.00	129.77	2.65
	F* & (P) value			1.92 (0.12)			3.77 (0.05)
Diastolic IV (mm Hg)	$\bar{x}$	83.23	85.21	87.02	86.72	86.38	3.15
	S.D.	10.11	12.15	12.16	9.75	11.43	
	$\bar{x}$ (age)	83.63	84.67	86.06	86.48	85.76	2.13
	F* & (P) value			1.43 (0.23)			3.57 (0.06)
Diastolic V (mm Hg)	$\bar{x}$	71.96	72.35	75.32	77.52	75.09	3.13
	S.D.	12.50	12.60	12.88	12.23	12.67	
	$\bar{x}$ (age)	72.54	71.55	74.00	77.05	74.17	1.62
	F* & (P) value			2.04 (0.11)			1.51 (0.22)
Systolic & Diastolic BP (IV & V)							
	F* & (P) value			1.61 (0.11)			1.92 (0.12)

$\bar{x}$ (age) = age and obesity adjusted mean.

Table 6-3: Systolic and Diastolic Blood Pressure Regressed on Log(Exposure Index), Age, Obesity Index, and Age · Log(Exposure Index)

Predictor	Estim. of Regression Coefficient	t	Pr[GT |t|]	Model R^2 $F_{4,378}$*	Incremental+ R^2 $F_{2,378}$*
Systolic BP					
(Intercept)	115.14	20.14	0.0001	0.125	0.033
Age	−0.183	−1.24	0.22	13.52	7.07
Log(Exp Ind)	−6.099	−2.47	0.01		
Age · Log(Exp Ind)	0.205	3.21	0.001		
Obesity Index	0.147	4.77	0.0001		
Diastolic BP (IV)					
(Intercept)	66.946	13.36	0.0001	0.091	0.009
Age	0.110	0.85	0.39	9.49	1.87
Log(Exp Ind)	−0.577	−0.27	0.79		
Age · Log(Exp Ind)	0.047	0.83	0.41		
Obesity Index	0.103	3.81	0.0002		
Diastolic BP (V)					
(Intercept)	50.435	9.34	0.0001	0.126	0.007
Age	0.193	1.39	0.17	13.63	1.56
Log(Exp Ind)	−1.175	−0.50	0.62		
Age · Log(Exp Ind)	0.060	0.99	0.32		
Obesity Index	0.122	4.17	0.0001		

*$\Pr[F_{4,200}$ GE $2.41] = 0.05$; $\Pr[F_{2,200}$ GE $3.04] = 0.05$

+R^2 due to log(exp. ind.) and age · log(exp. ind.)

Systolic BP & Diastolic BP (IV & V)	F	P
Age	1.91	0.13
Log(Exp Ind)	2.68	0.05
Age · Log(Exp Ind)	4.08	0.01
Obesity Index	10.06	0.0001

Table 6-4: "Coronary" Electrocardiograms
By Current Exposure Category
(Comparison versus Exposed and Comparison versus DL, M, DH)

	Comparison N(%)	DL N(%)	M N(%)	DH N(%)	All Exposed N(%)
Q and QS Pattern					
1. Normal	228(98.7)	47(100)	64(100)	44(100)	155(100)
2. Class I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
3. Class II	1(0.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
4. Class III	2(0.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
S-T Junction and Segment					
1. Normal	229(98.7)	47(100)	60(95.2)	42(95.4)	149(96.7)
2. Junct Depression:					
a. LT 0.5mm	1(0.4)	0(0.0)	1(1.6)	1(2.3)	2(1.3)
b. 0.5mm - 0.9mm	2(0.9)	0(0.0)	1(1.6)	1(2.3)	2(1.3)
c. 1.0mm or more	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
3. Elevation GE 1.0mm	0(0.0)	0(0.0)	1(1.6)	0(0.0)	1(0.7)
T-Wave					
1. Normal	231(99.6)	47(100)	62(96.9)	42(95.5)	151(97.4)
2. Amplitude LT 5 mm	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
3. Amplitude 1-5 mm	0(0.0)	0(0.0)	2(3.1)	0(0.0)	2(1.3)
4. Flat or Small	1(0.4)	0(0.0)	0(0.0)	2(4.6)	2(1.3)
5. Diphasic	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 6-7: Heart Rate Regressed on Age, Log(Exposure Index), Hemoglobin, Age · Log(Exposure Index), and Hemoglobin · Log(Exposure Index)

Predictor	Estim. of Regression Coefficient	t	Pr[GT $\lvert t\rvert$]	Model R^2 $F_{5,330}$*	Incremental+ R^2 $F_{3,330}$*
(Intercept)	59.8759	7.42	0.0001	0.064	0.0493
Age	0.1658	1.22	0.22	4.54	5.83
Log(Exp Ind)	3.6432	0.85	0.40		
Age · Log(Exp Ind)	-0.1624	-2.68	0.008		
Hemoglobin	0.0868	0.20	0.84		
Hemoglobin · Log(Exp Ind)	0.2753	1.19	0.24		

*Pr[$F_{5,200}$ GE 2.26] = 0.05; Pr[$F_{3,200}$ GE 2.65] = 0.05
+R^2 due to log(exp. ind.), age · log(exp. ind.), and hemoglobin · log(exp. ind.)

Table 6-5: Systolic Time Interval
By Current Exposure Category
(Comparison versus DL, M, DH, and All Exposed))

	Comparison N = 207	DL N = 38	M N = 52	DH N = 35	Exposed N = 125	Exposed - Comparison
QS_2 - $\bar{x}$	392.96	389.61	384.60	390.86	387.87	-5.09
S.D.	21.46	27.90	29.34	30.02	29.01	
$\bar{x}$ (adj.)	391.56	392.29	387.26	392.19	390.19	-1.37
F & (P) value			1.22 (0.30)		0.55	(0.46)
LVET - $\bar{x}$	292.31	289.61	288.10	288.91	288.78	-3.52
S.D.	16.75	25.45	21.03	22.07	22.57	
$\bar{x}$ (adj.)	291.14	291.82	290.33	290.07	290.72	-0.42
F & (P) value			0.21 (0.89)		0.10	(0.76)
PEP - $\bar{x}$	100.65	100.00	96.50	101.94	99.09	-1.56
S.D.	12.88	9.01	14.79	14.43	13.29	
$\bar{x}$ (adj.)	100.42	100.47	96.94	102.12	99.46	-0.96
F & (P) value			1.44 (0.23)		0.41	(0.52)
PEP/LVET - $\bar{x}$	0.35	0.35	0.34	0.35	0.34	-0.01
S.D.	0.05	0.04	0.05	0.05	0.05	
$\bar{x}$ (adj.)	0.35	0.35	0.33	0.35	0.34	-0.01
F & (P) value			1.02 (0.38)		0.18	(0.86)
Heart rate - $\bar{x}$	66.79	70.08	70.65	70.14	70.34	3.37
S.D.	9.56	11.76	12.89	10.47	11.82	
F & (P) value			2.70 (0.05)		2.69	(0.01)
QS_2 & LVET* t & (P) value			0.86 (0.52)		-0.29	(0.75)

$\bar{x}$ (adj.) = mean, adjusted for age and heart rate

PEP/LVET is dimensionless; rate is beats per minute; all other units are milliseconds.

* Dependent variables used in multivariate analysis of covariance

Table 6-6: Systolic Time Intervals Regressed on Log(Exposure Index), Age, Age · Log(Exposure Index) Heart Rate, and Heart Rate · Log(Exposure Index)

	Predictor	Estim. of Regression Coefficient	t	Pr[GT |t|]	Model R^2 $F_{5,344}$*	Incremental+ R^2 $F_{2,344}$*
QS_2	(Intercept)	501.11	33.82	0.0001	0.6105	0.0055
	Age	0.11	0.57	0.57	107.85	1.62
	Heart Rate	-1.67	-8.59	0.0001		
	Log(Exp Ind)	-4.84	-0.65	0.52		
	Age·Log(Exp Ind)	0.19	2.09	0.04		
	Heart Rate · Log(Exp Ind)	-0.03	-0.36	0.72		
LVET	(Intercept)	377.60	35.13	0.0001	0.6649	0.0044
	Age	0.12	0.85	0.40	136.50	1.51
	Heart Rate	-1.33	-9.47	0.0001		
	Log(Exp Ind)	-1.08	-0.20	0.84		
	Age·Log(Exp Ind)	0.12	1.85	0.07		
	Heart Rate · Log(Exp Ind)	-0.05	-0.73	0.47		
PEP	(Intercept)	123.51	10.23	0.0001	0.0750	0.0025
	Age	-0.01	-0.06	0.96	5.57	0.31
	Heart Rate	-0.33	-2.11	0.04		
	Log(Exp Ind)	-3.77	-0.62	0.54		
	Age·Log(Exp Ind)	0.07	0.92	0.36		
	Heart Rate · Log(Exp Ind)	0.01	0.20	0.84		
$\frac{\text{PEP}}{\text{LVET}}$	(Intercept)	31.62	6.85	0.0001	0.0207	0.0006
	Age	-0.02	-0.28	0.78	1.45	0.08
	Heart Rate	0.05	0.88	0.38		
	Log(Exp Ind)	-0.92	-0.39	0.70		
	Age·Log(Exp Ind)	0.01	0.33	0.74		
	Heart Rate · Log(Exp Ind)	0.01	0.25	0.80		

*Pr[$F_{5,200}$ GE 2.26] = 0.05; Pr[$F_{2,200}$ GE 3.04] = 0.05

+R^2 due to log(exp. ind.) and age · log(exp. ind.)

QS2 & LVET	F	P
Age	0.36	0.70
Heart Rate	51.49	0.0001
Log(Exp Ind)	0.23	0.79
Age·Log(Exp Ind)	2.47	0.09
Heart Rate·Log(Exp Ind)	0.27	0.76

Table 6-10: Trace Minerals By Current Exposure Category
(Comparison Versus DL, M, DH, and All Exposed)

Comparison			DL	M	DH	All Exposed
Serum	N	213	42	59	42	143
Zn	$\bar{x}$	125.8	127.5	125.9	131.1	127.9
	S.D.	22.27	19.60	25.60	49.06	32.84
	F* & (P) values		0.47 (0.71)			-0.66(0.51)
Serum	N	213	42	59	42	143
Cu	$\bar{x}$	99.4	100.8	99.9	103.6	101.3
	S.D.	20.51	17.12	21.53	21.95	20.40
	F* & (P) values		0.53 (0.67)			0.86(0.39)
Urine	N	13	0	11	11	22
Zn	$\bar{x}$	0.49	—	0.72	0.64	0.68
	S.D.	0.30	—	0.32	0.35	0.33
	F* & (P) values		1.64 (0.21)			-1.72(0.09)
Blood	N	214	44	61	44	149
Lead	$\bar{x}$	8.39	12.89	13.69	17.02	14.44
(mcg/dl)	S.D.	8.17	8.64	10.32	10.33	9.94
	t & (P) values		15.36 (LT 0.01)			-6.12(LT0.01)

Table 6-9: Cholesterol, Triglycerides, High Density Lipoproteins, Low Density Lipoproteins, and Ratio of Low to High Density Lipoproteins, Regressed on Log(Exposure Index), Age, and Age · Log(Exposure Index)

Predictor	Estim. of Regression Coefficient	t	Pr[GT |t|]	Model R^2 $F_{3,374}$*	Incremental+ R^2 $F_{2,374}$*
Cholesterol					
(Intercept)	101.64	6.66	0.0001	0.153	0.027
Age	2.769	6.30	0.0001	22.47	5.95
Log(Exp Ind)	23.699	3.27	0.001		
Age · Log(Exp Ind)	-0.644	-3.45	0.0006		
Triglycerides					
(Intercept)	22.539	0.58	0.56	0.035	0.028
Age	4.135	3.68	0.0003	4.55	5.48
Log(Exp Ind)	48.732	2.63	0.009		
Age · Log(Exp Ind)	-1.491	-3.12	0.002		
HDL++					
(Intercept)	42.1952	9.23	0.0001	0.022	0.007
Age	0.1772	1.35	0.18	2.82	1.28
Log(Exp Ind)	-1.2816	-0.55	0.58		
Age · Log(Exp Ind)	0.0034	0.06	0.95		
LDL++					
(Intercept)	53.5286	3.94	0.0001	0.120	0.015
Age	1.8439	4.70	0.0001	16.58	3.18
Log(Exp Ind)	16.2922	2.51	0.01		
Age · Log(Exp Ind)	-0.3857	-2.32	0.02		
LDL/HDL					
(Intercept)	1.2917	2.99	0.003	0.044	0.017
Age	0.0373	2.99	0.003	5.56	3.23
Log(Exp Ind)	0.5080	2.47	0.01		
Age · Log(Exp Ind)	-0.0114	-2.15	0.03		

*Pr[$F_{3,200}$ GE 2.65] = 0.05; Pr[$F_{2,200}$ GE 3.04] = 0.05

+R^2 due to log(exp. ind.) & age · log(exp. ind.)

++F statistics for R^2 will be $F_{3,364}$ and $F_{2,364}$.

Cholesterol, Triglycerides, HDL, & LDL	F	P
Age	10.21	0.0001
Log(Exp Ind)	2.53	0.04
Age · Log(Exp Ind)	3.14	0.01

Table 6-8: Serum Lipids By Current Exposure Category (Comparison Versus DL, M, DH, and All Exposed))

Comparison			DL	M	DH	All Exposed	Difference (Exposed - Comparison)
Chol.	N	214	40	60	43	143	
	$\bar{x}$	196.06	196.58	202.02	207.37	202.10	6.04
	S.D.	38.91	32.93	41.38	40.25	38.96	
	$\bar{x}$ (age)	198.74	193.99	197.91	202.16	198.07	0.69
	F* & (P) value			0.36 (0.79)		0.03 (0.86)	
Trigly-cerides	N	214	40	60	43	143	
	$\bar{x}$	159.25	164.60	149.13	133.33	148.71	10.54
	S.D.	98.23	110.59	80.64	57.98	94.84	
	$\bar{x}$ (age)	161.19	162.72	146.16	129.55	145.90	15.22
	F* & (P) value			1.62 (0.18)		2.19 (0.14)	
HDL	N	214	40	60	43	143	
	$\bar{x}$	46.45	45.20	47.08	47.79	46.77	0.32
	S.D.	11.33	10.58	11.46	8.90	10.48	
	$\bar{x}$ (age)	46.74	44.92	46.64	47.23	46.33	0.42
	F* & (P) value			0.37 (0.78)		0.12 (0.73)	
LDL	N	208	37	60	43	140	
	$\bar{x}$	118.75	120.62	125.95	132.91	126.68	7.93
	S.D.	34.30	28.82	33.48	37.84	33.85	
	$\bar{x}$ (age)	120.86	118.33	122.86	128.99	123.52	2.65
	F* & (P) value			0.91 (0.56)		0.52 (0.47)	
LDL/HDL	N	208	37	60	43	140	
	$\bar{x}$	2.70	2.83	2.82	2.87	2.84	0.14
	S.D.	1.06	1.12	1.00	0.96	1.01	
	$\bar{x}$ (age)	2.73	2.79	2.78	2.82	2.79	0.06
	F* & (P) value			0.11 (0.95)		0.30 (0.58)	
Cholesterol, Triglycerides, HDL, & LDL	F* & (P) value			1.18 (0.29)		1.30 (0.27)	

$\bar{x}$ (age) = age adjusted mean.
*F test for differences among adjusted means.

Figure 6-1: Scatter Plot of Systolic Blood Pressure vs. Log(exp. ind.)

Figure 6-2: Scatter Plot of Diastolic Blood Pressure IV vs. Log(exp. ind.)

Figure 6-3: Scatter Plot of Diastolic Blood Pressure V vs. Log(exp. ind.)

Figure 6-4: Scatter Plot of QS_2 vs. Log(exp. ind.)

Figure 6-5: Scatter Plot of LVET vs. Log(exp. ind.)

Figure 6-6: Scatter Plot of PEP vs. Log(exp. ind.)

Figure 6-7: Scatter Plot of PEP/LVET vs. Log(exp. ind.)

Figure 6-8: Scatter Plot of Heart Rate vs. Log(exp. ind.)

Figure 6-9: Scatter Plot of Serum Cholesterol vs. Log(exp. ind.)

Figure 6-10: Scatter Plot of Serum Triglyceride vs. Log(exp. ind.)

Figure 6-11: Scatter Plot of Serum High Density Lipoprotein (HDL) vs. Log(exp. ind.)

Figure 6-12: Scatter Plot of Serum Low Density Lipoprotein (LDL) vs. Log(exp. ind.)

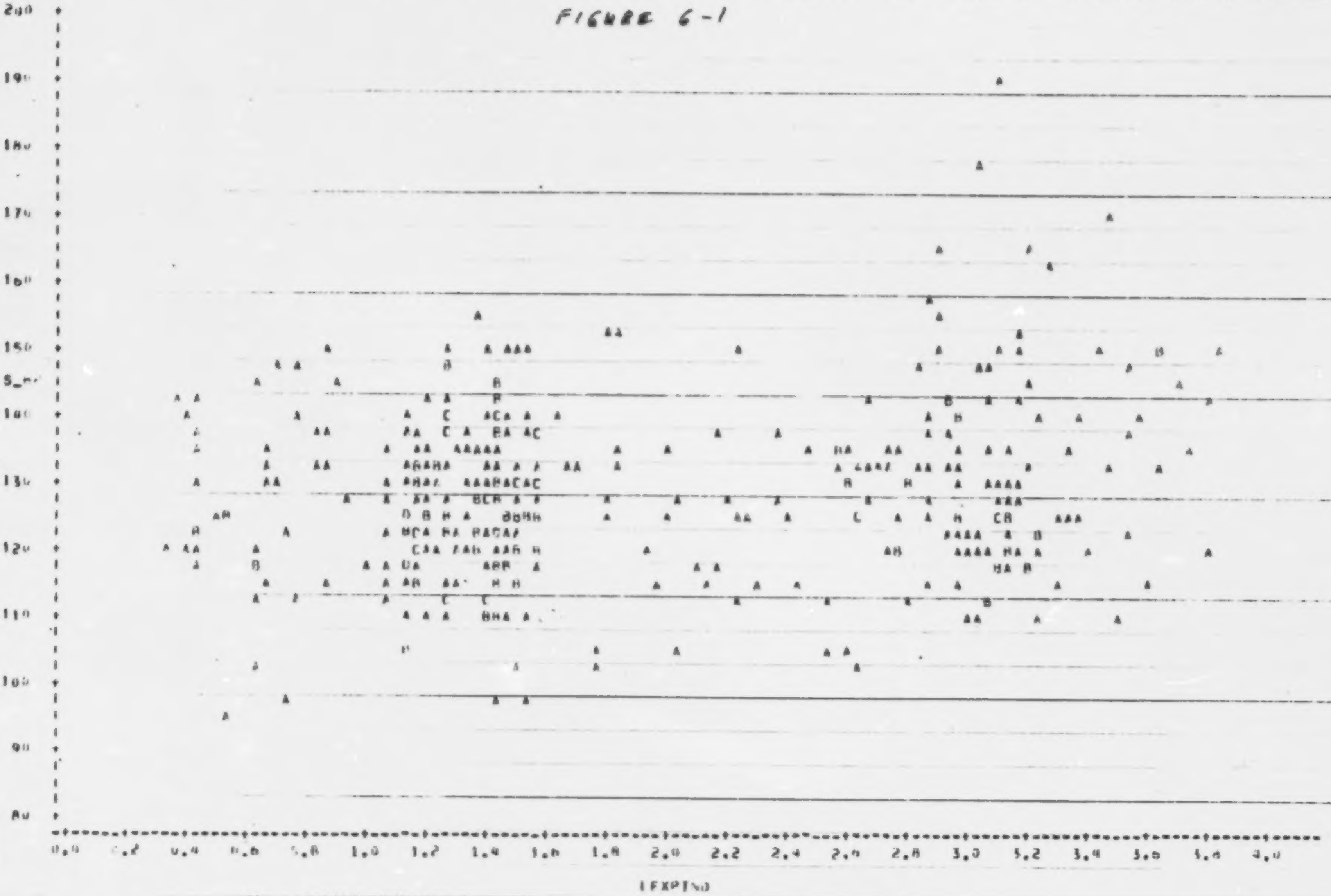
Figure 6-13: Scatter Plot of Serum LDL/HDL Ratios vs. Log(exp. ind.)

STATISTICAL ANALYSIS SYSTEM 2

09:15 [illegible], [illegible] 21, 1981

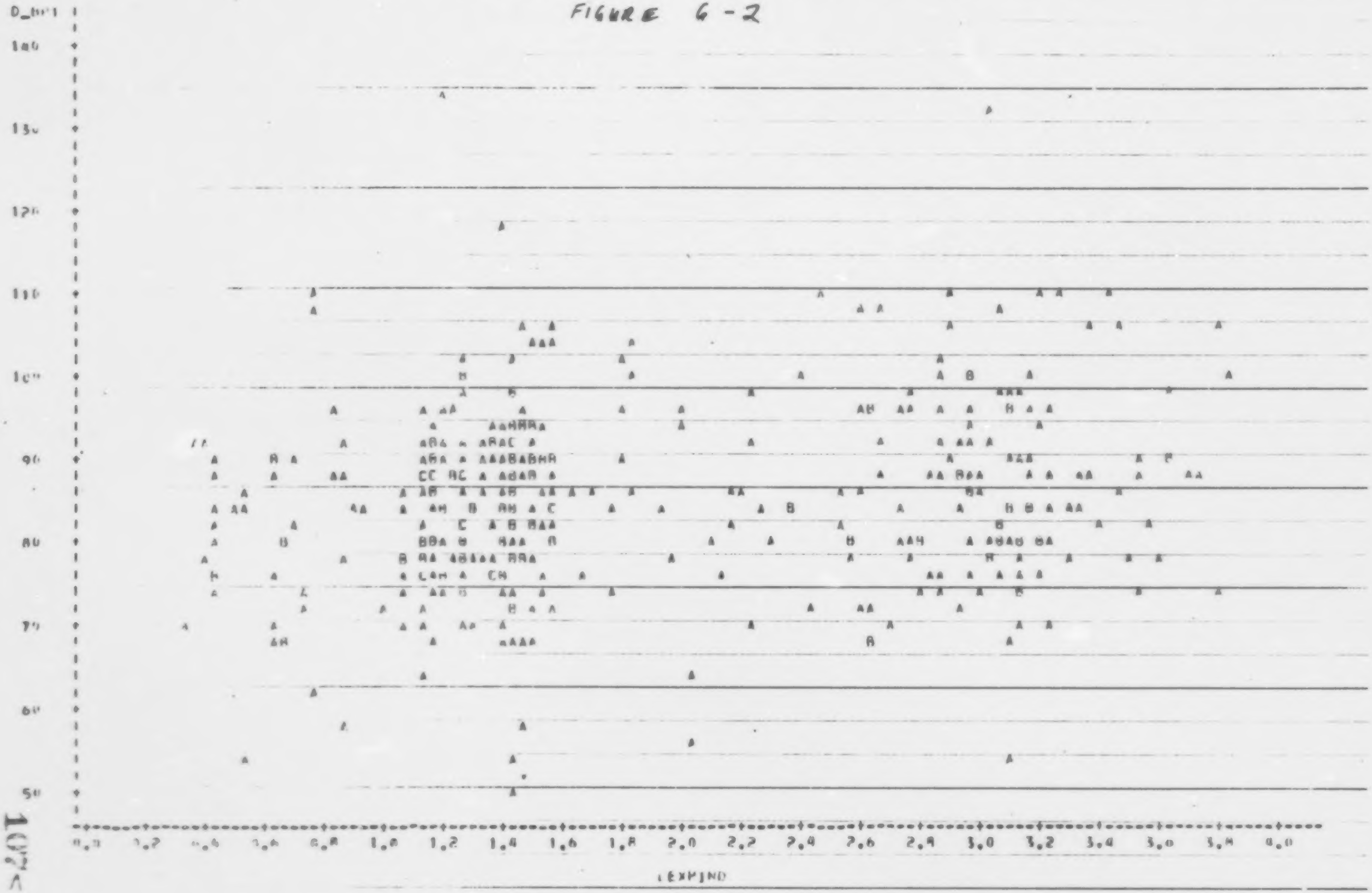
PLOT OF S_[illegible]*LEXPTNO   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-1



PLOT OF D_HPT*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-2

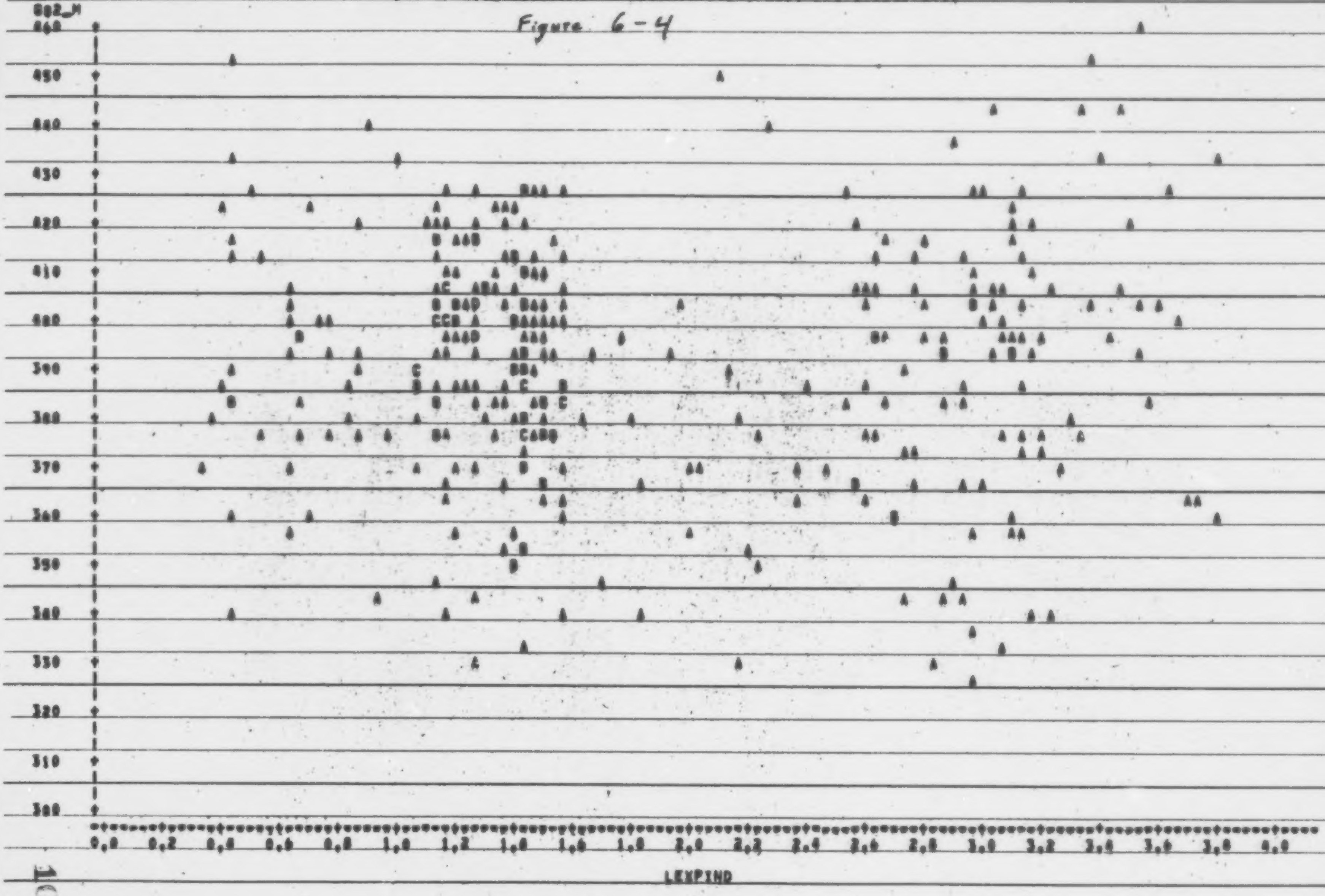


STATISTICAL ANALYSIS SYSTEM 1

15:05 THURSDAY, NOVEMBER 12, 1981

PLOT OF 002_M*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

Figure 6-4

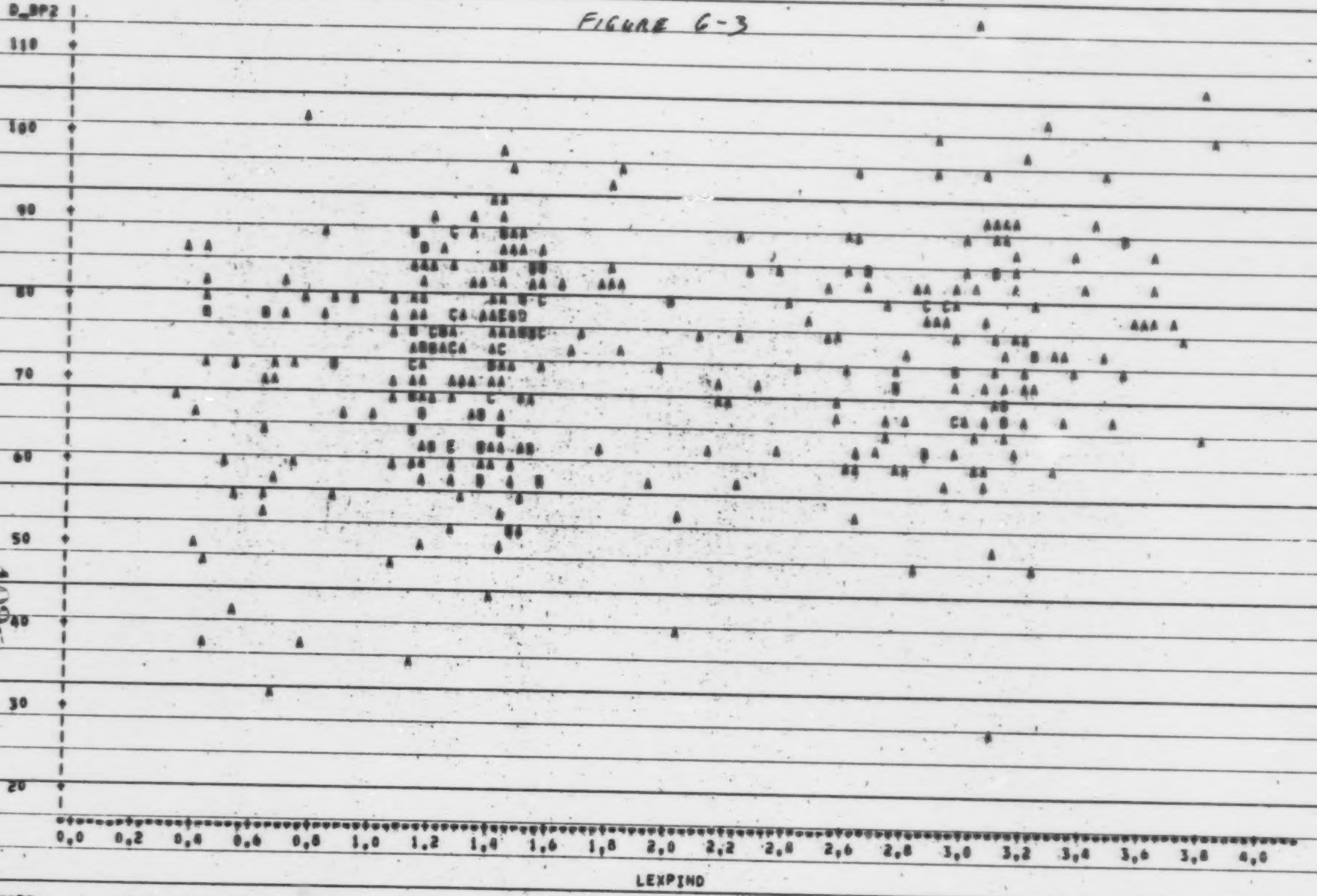


S T A T I S T I C A L   A N A L Y S I S   S Y S T E M

9:15 WEDNESDAY, OCTOBER 21, 1981

PLOT OF D_BP2*LEXPIND   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-3



NOTE: 1 OBS HAD MISSING VALUES

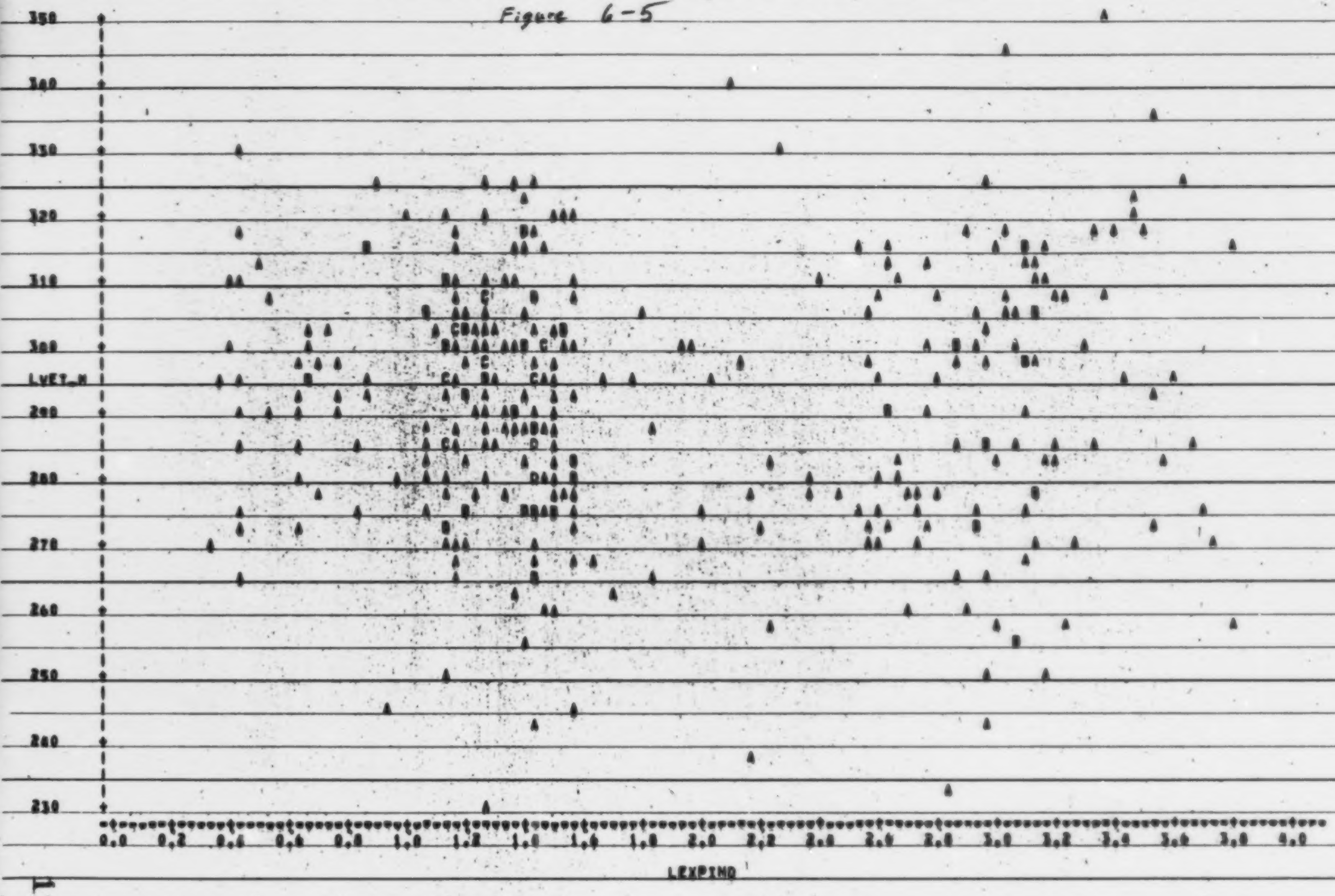
STATISTICAL ANALYSIS SYSTEM

3

15:05 THURSDAY, NOVEMBER 12, 1981

PLOT OF LVET_M*LEXPIND   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

Figure 6-5

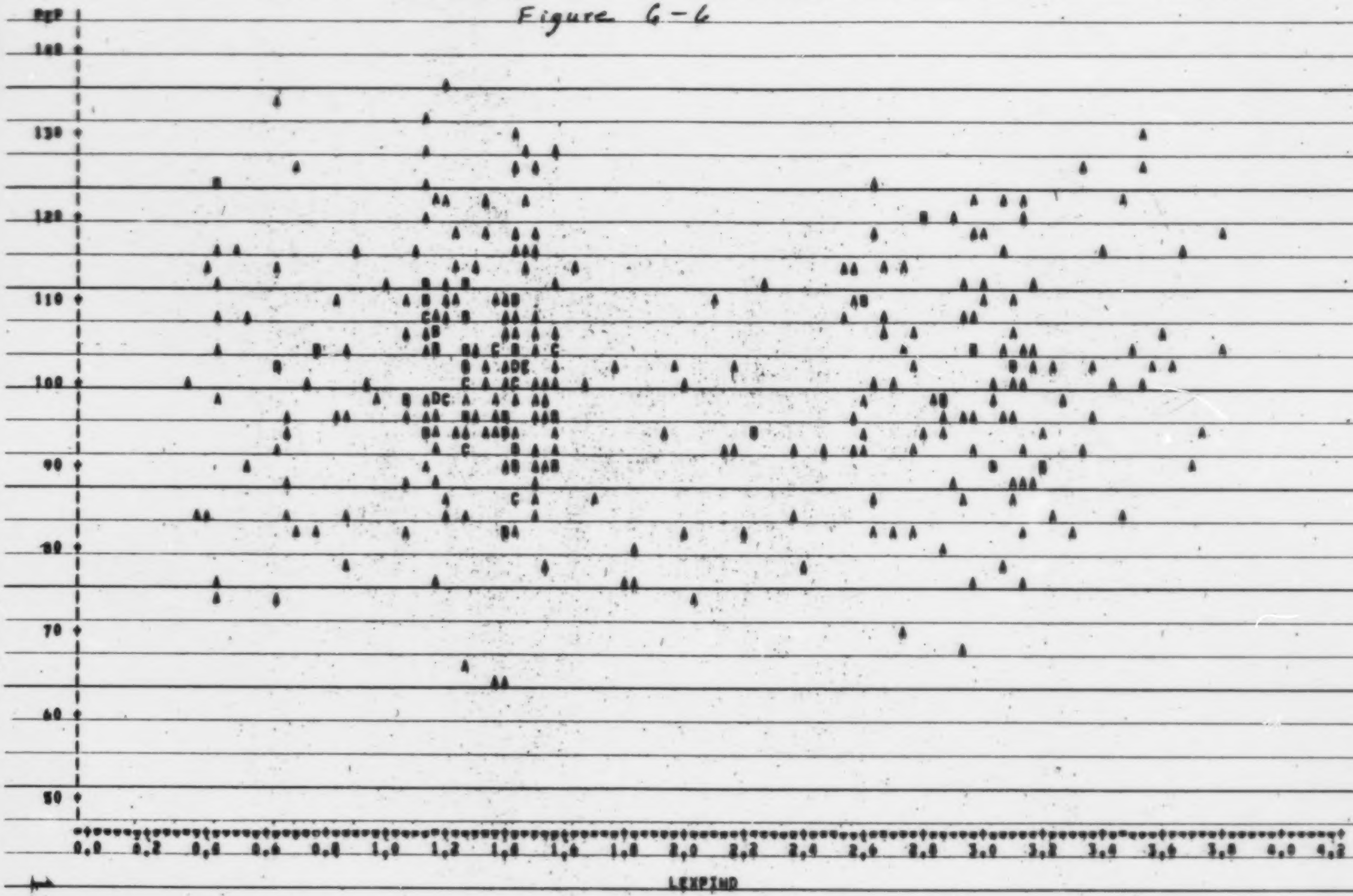


STATISTICAL ANALYSIS SYSTEM

15:05 THURSDAY, NOVEMBER 12, 1981

PLOT OF PEP*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

Figure 6-6

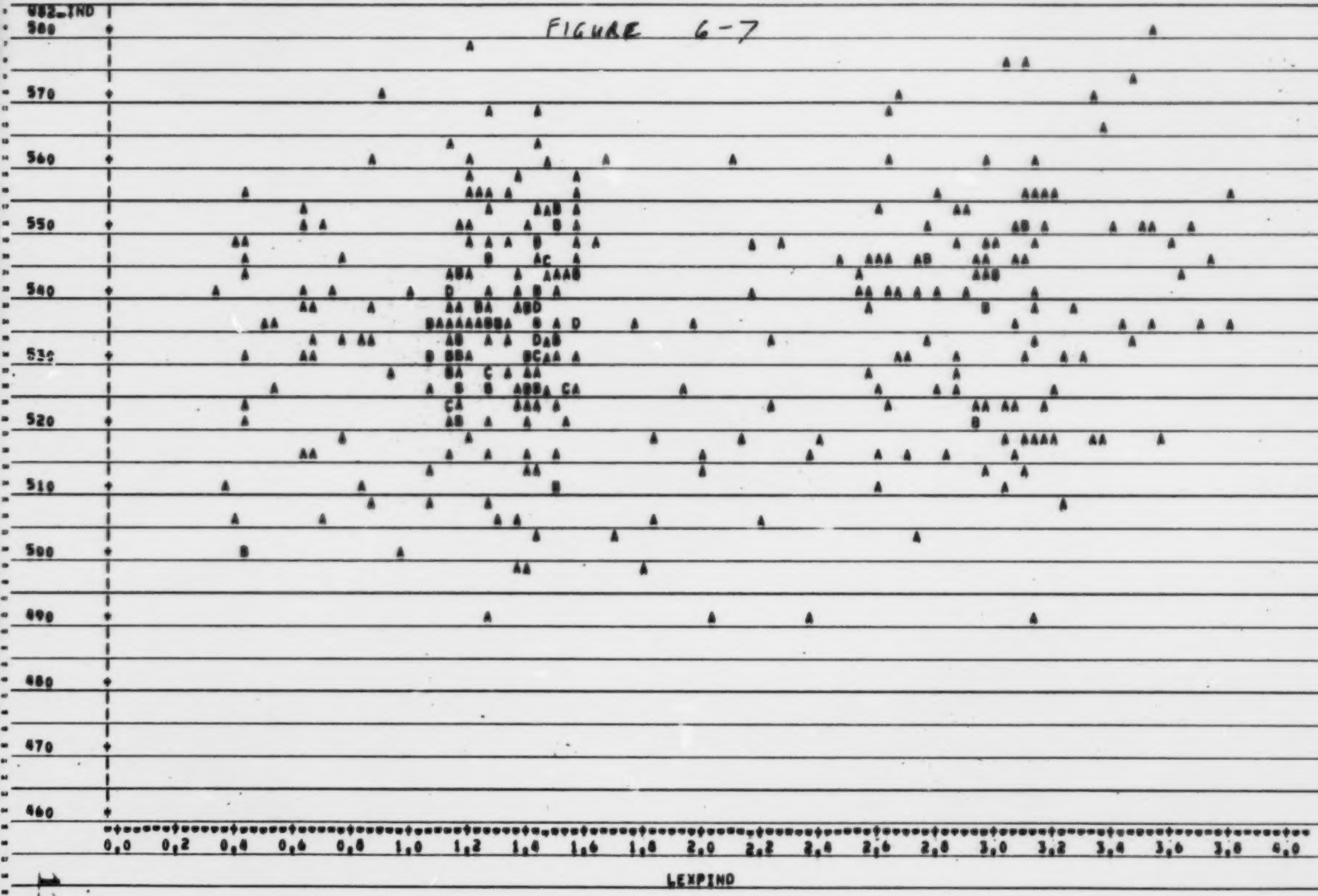


6-7

S T A T I S T I C A L   A N A L Y S I S   S Y S T E M   13:56 FRIDAY, JULY 17, 1981   1

PLOT OF U82_IND*LEXPIND   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-7



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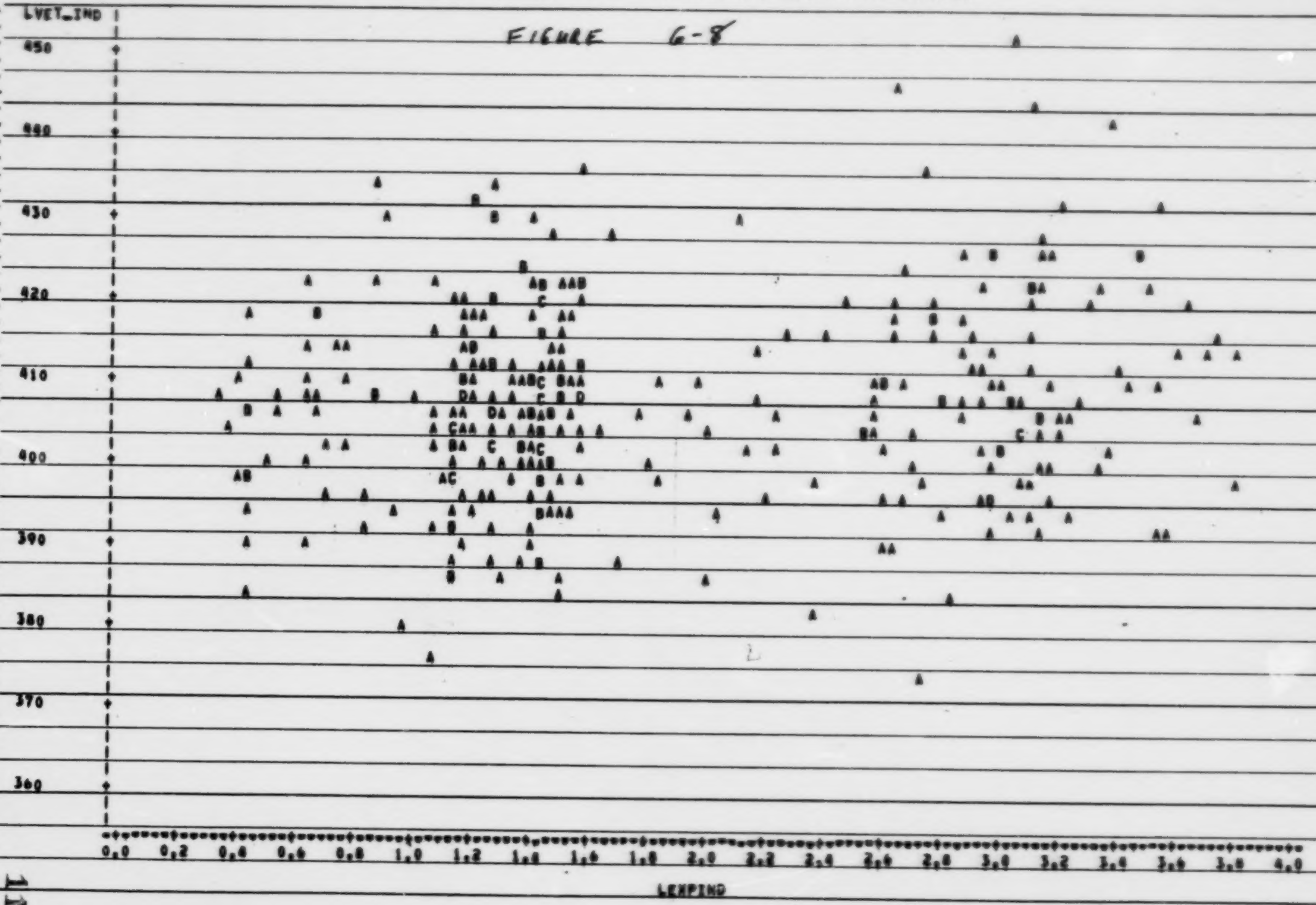
6-8

S T A T I S T I C A L   A N A L Y S I S   S Y S T E M   13:56 FRIDAY, JULY 17, 1981   2

PLOT OF LVET_IND*LEXPIND   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-8

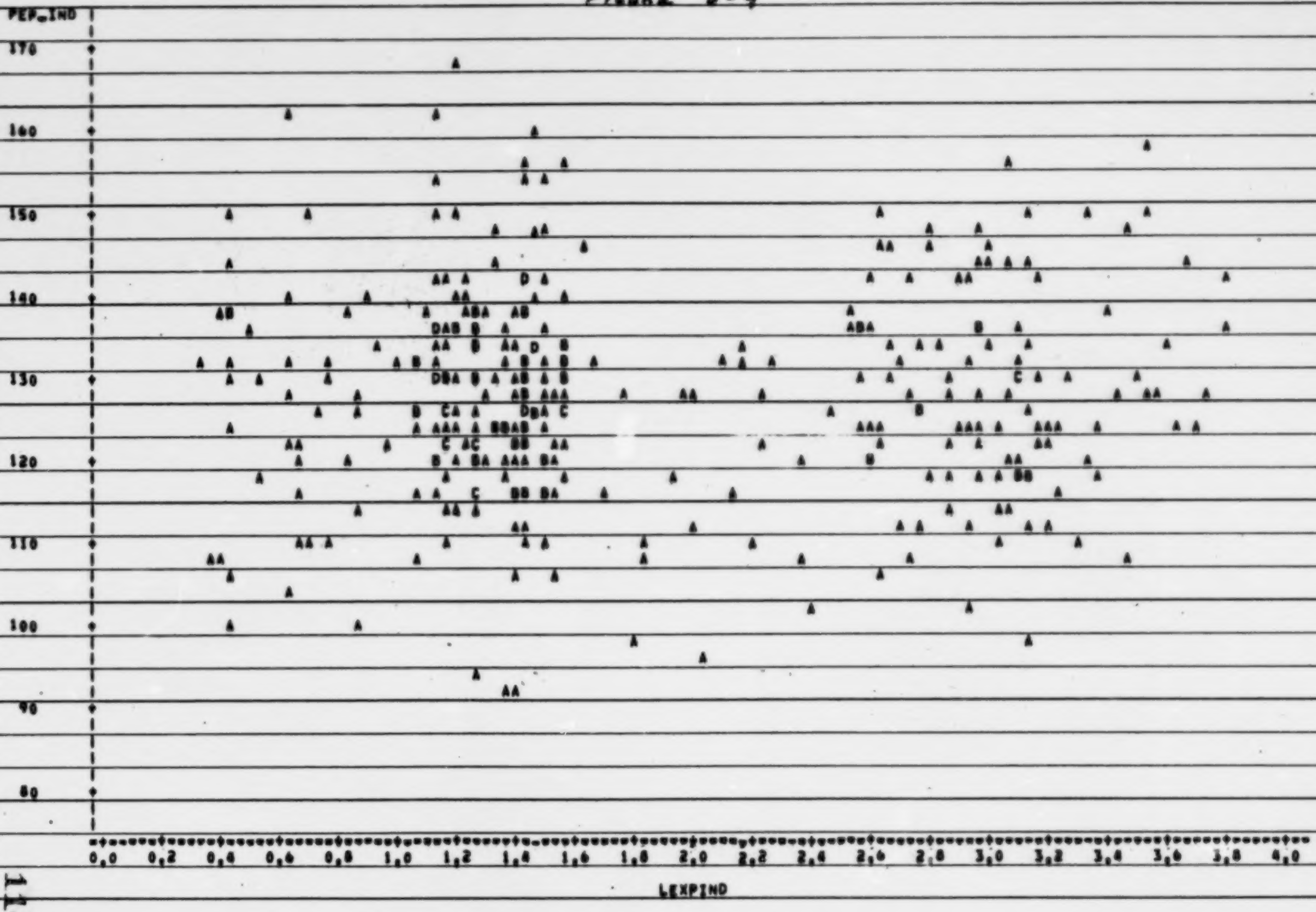


6 -

STATISTICAL ANALYSIS SYSTEM 13:56 FRIDAY, JULY 17, 1981 3

PLOT OF PEP_IND*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-9

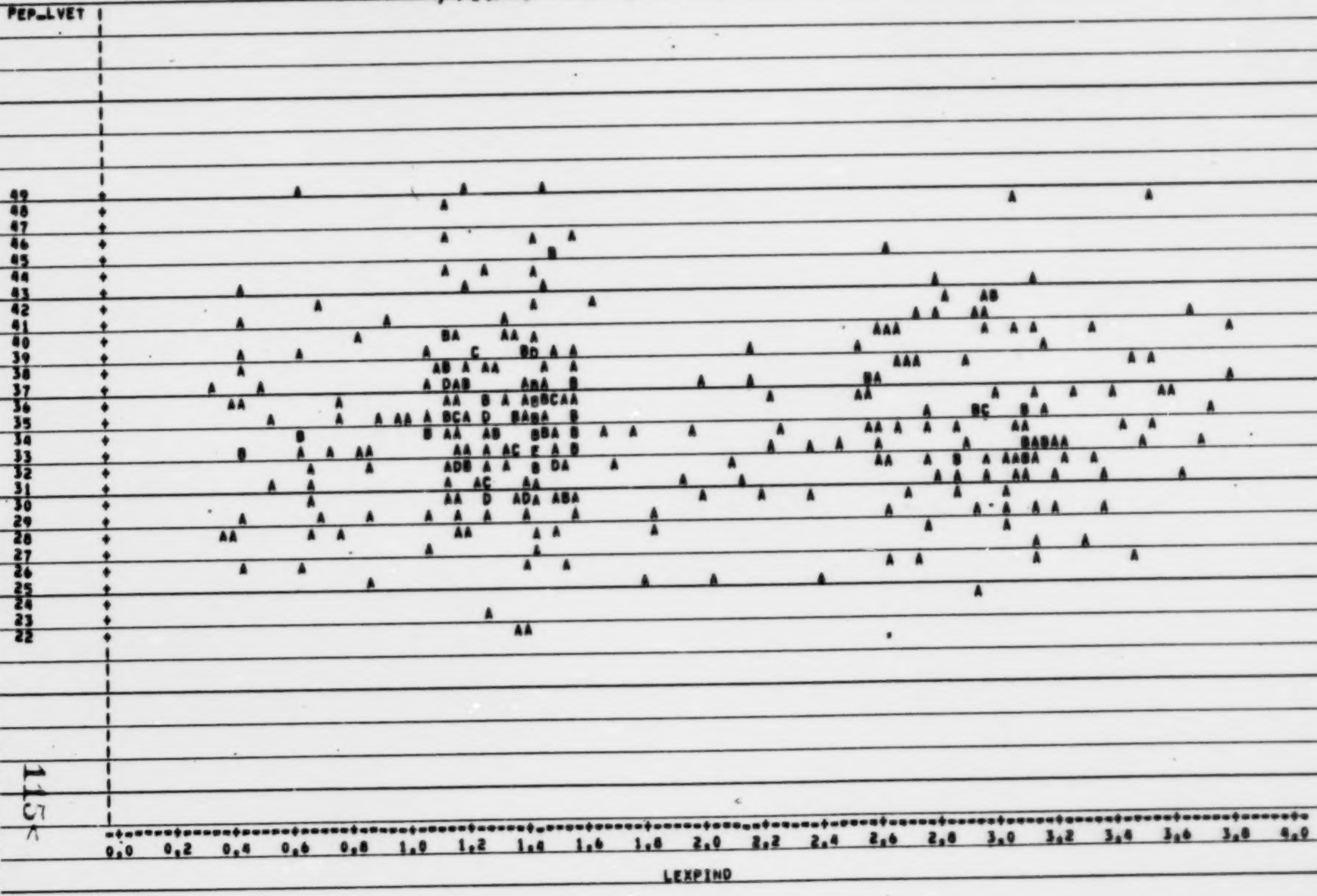


64

STATISTICAL ANALYSIS SYSTEM 13:56 FRIDAY, JULY 17, 1981 4

PLOT OF PEP_LVET*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

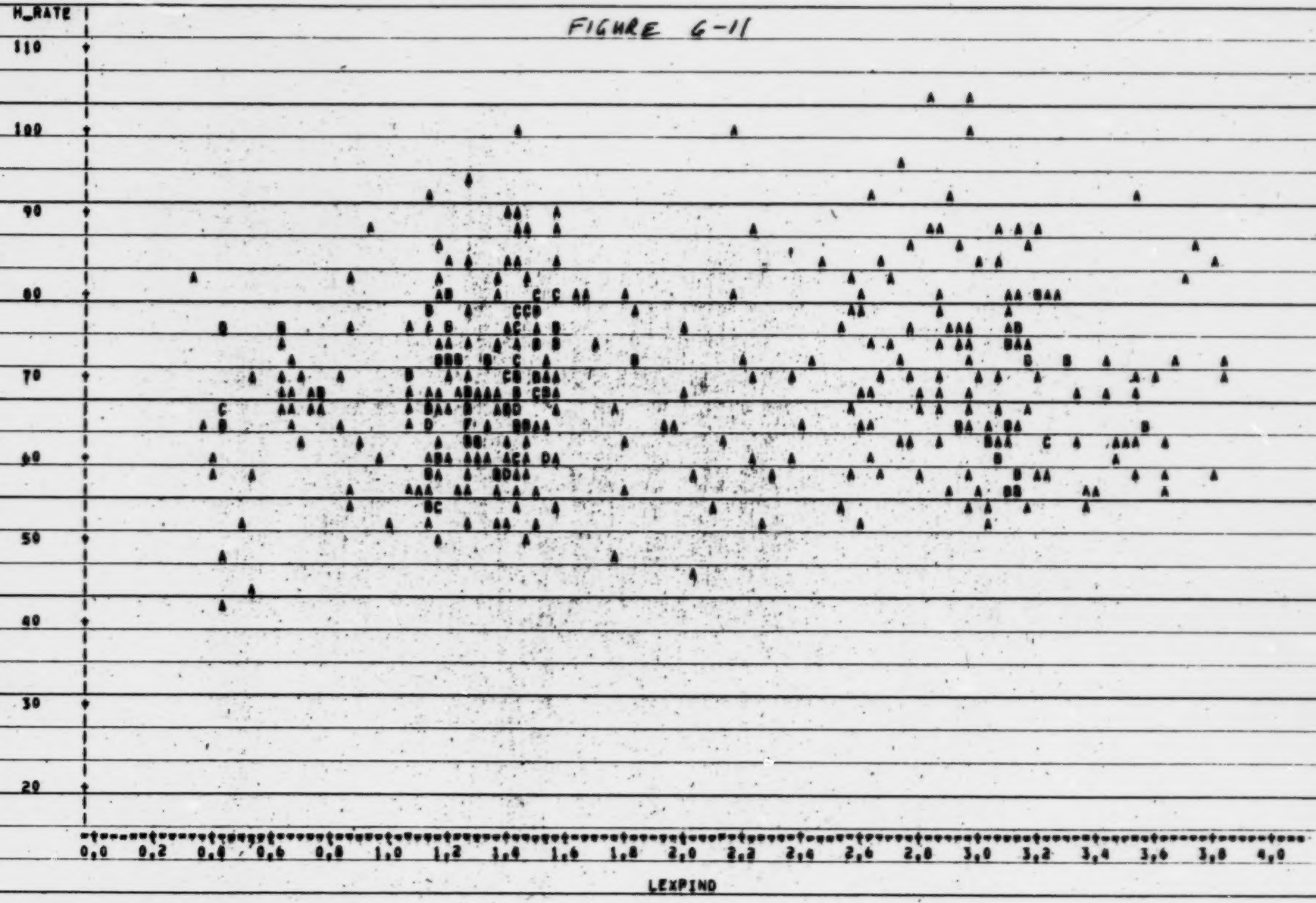
FIGURE C-10



S T A T I S T I C A L   A N A L Y S I S   S Y S T E M   16:22 TUESDAY, OCTOBER 20, 1981   1

PLOT OF H_RATE*LEXPIND   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-11

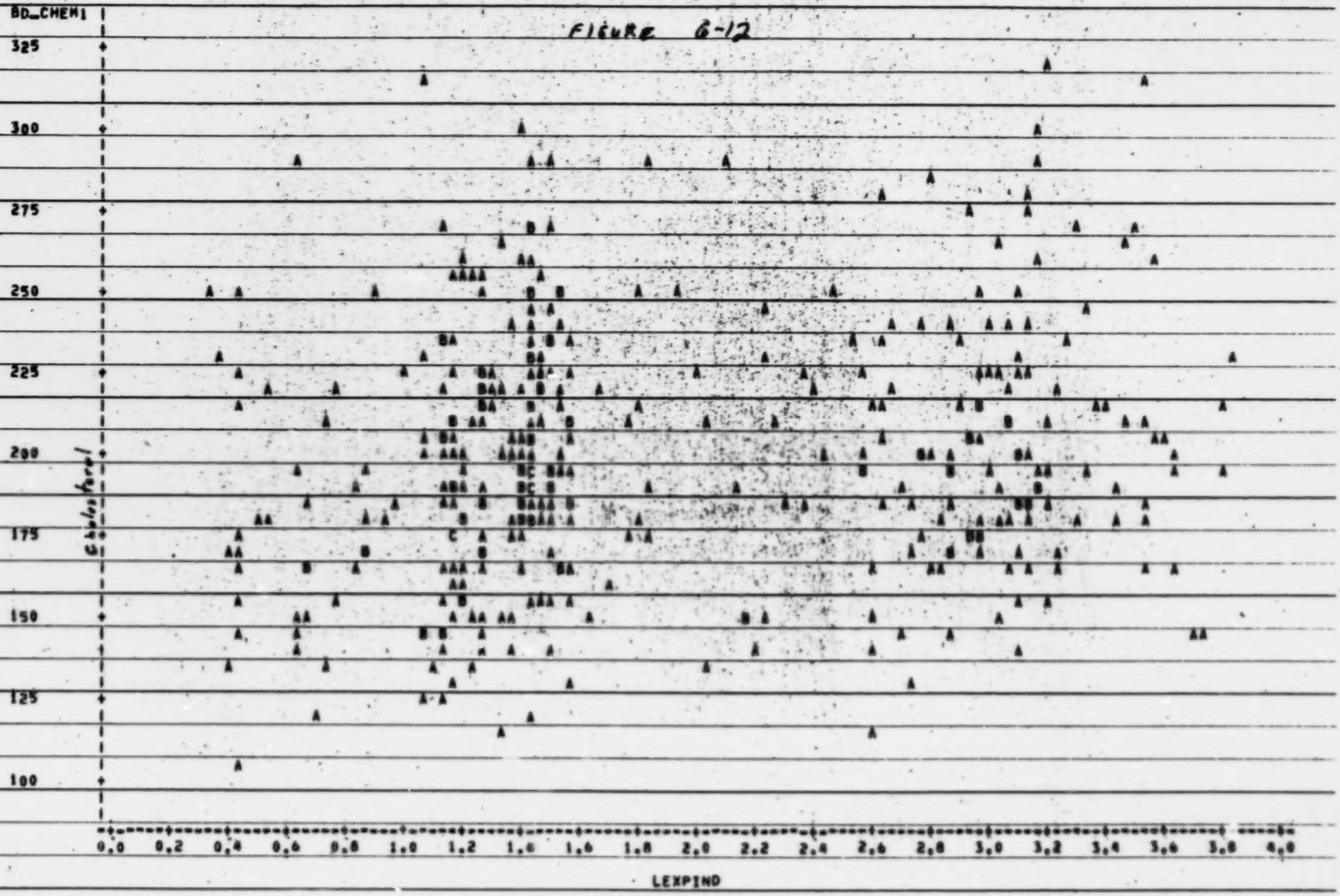


NOTE: 1 OBS HAD MISSING VALUES

STATISTICAL ANALYSIS SYSTEM 16:35 TUESDAY, OCTOBER 20, 1981 1

PLOT OF BD_CHEM1*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-12

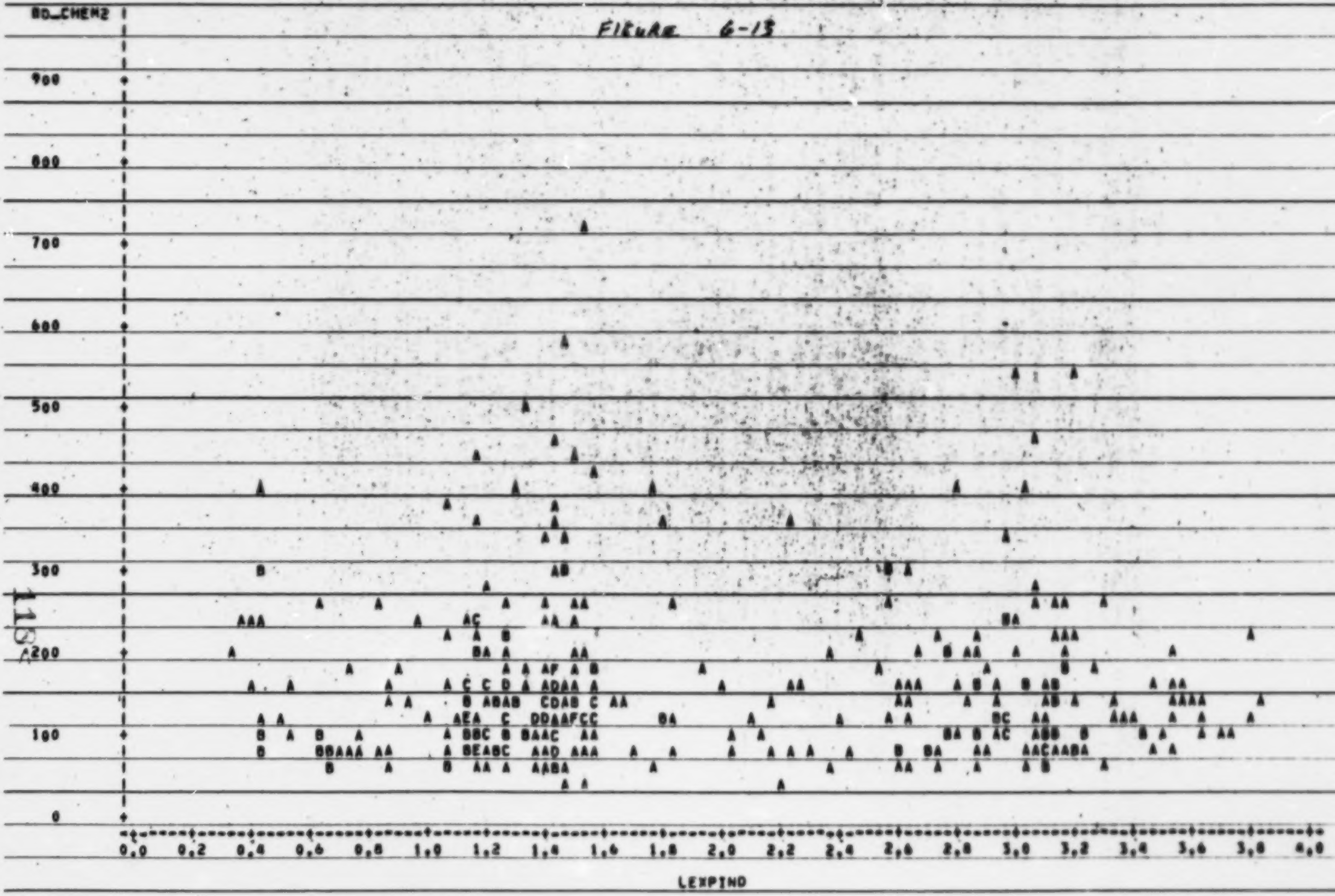


NOTE: 22 OBS HAD MISSING VALUES

STATISTICAL ANALYSIS SYSTEM 16:35 TUESDAY, OCTOBER 20, 1981 2

PLOT OF BD_CHEM2*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

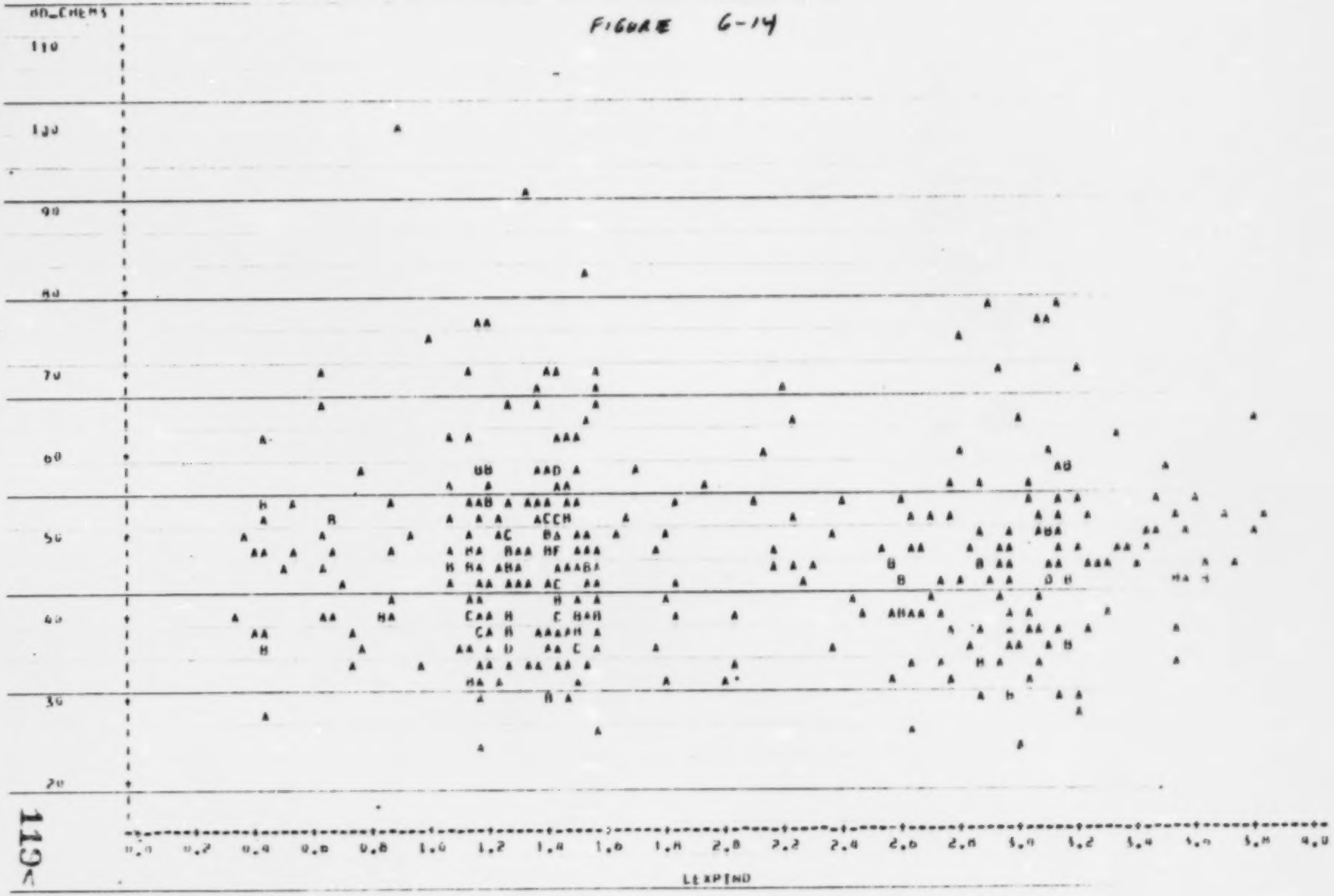
FIGURE 6-13



NOTE: 22 OBS HAD MISSING VALUES

PLOT OF [illegible]_CHEM*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE G-14



NOTE: 22 OBS HAD MISSING VALUES

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                        S T A T I S T I C A L   A N A L Y S I S   S Y S T E M     16:35 TUESDAY, OCTOBER 20, 1981    4

                   PLOT OF BD_CHEM4*LEXPIND    LEGEND: A = 1 OBS, B = 2 OBS, ETC.
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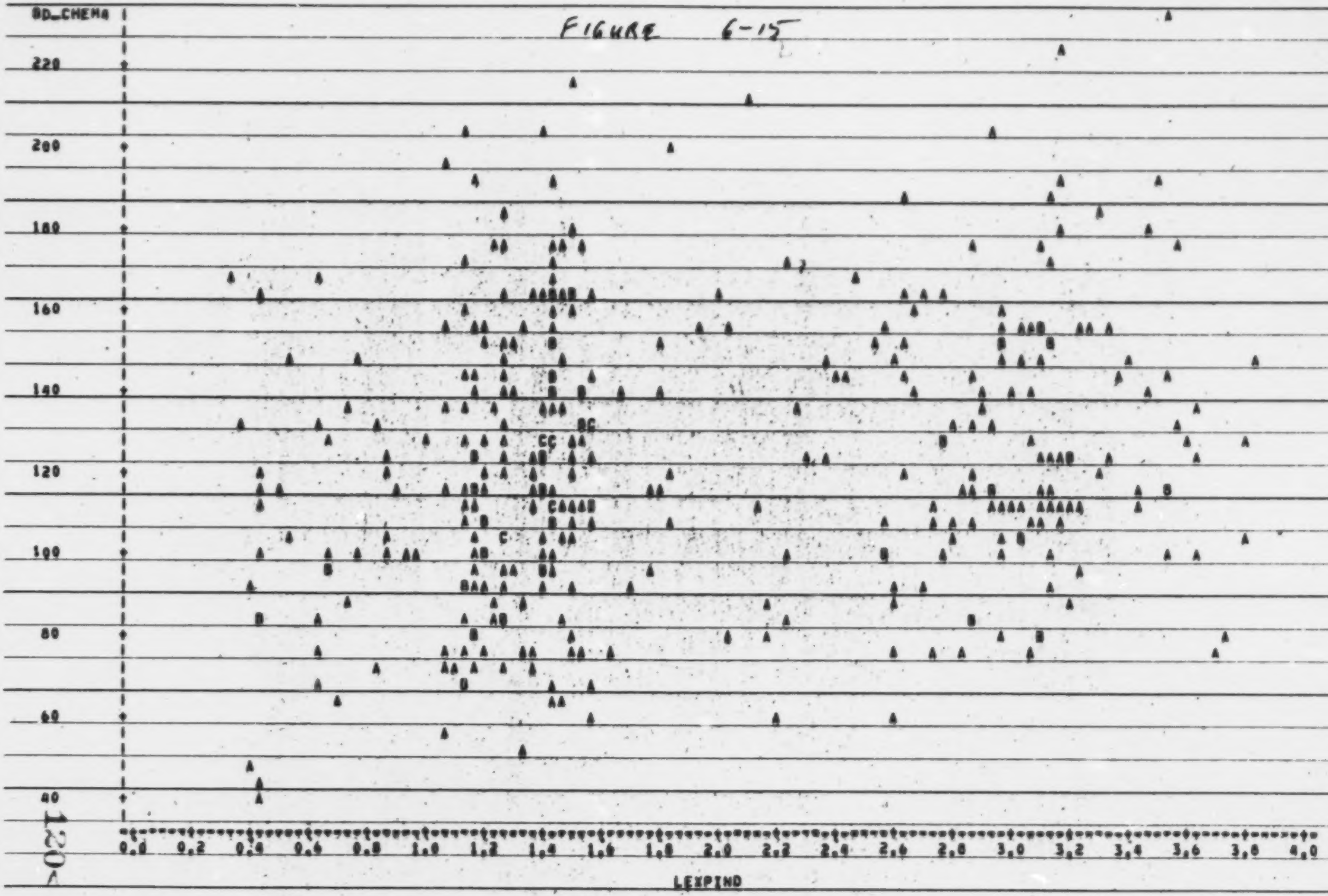
FIGURE 6-15

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NOTE:   32 OBS HAD MISSING VALUES
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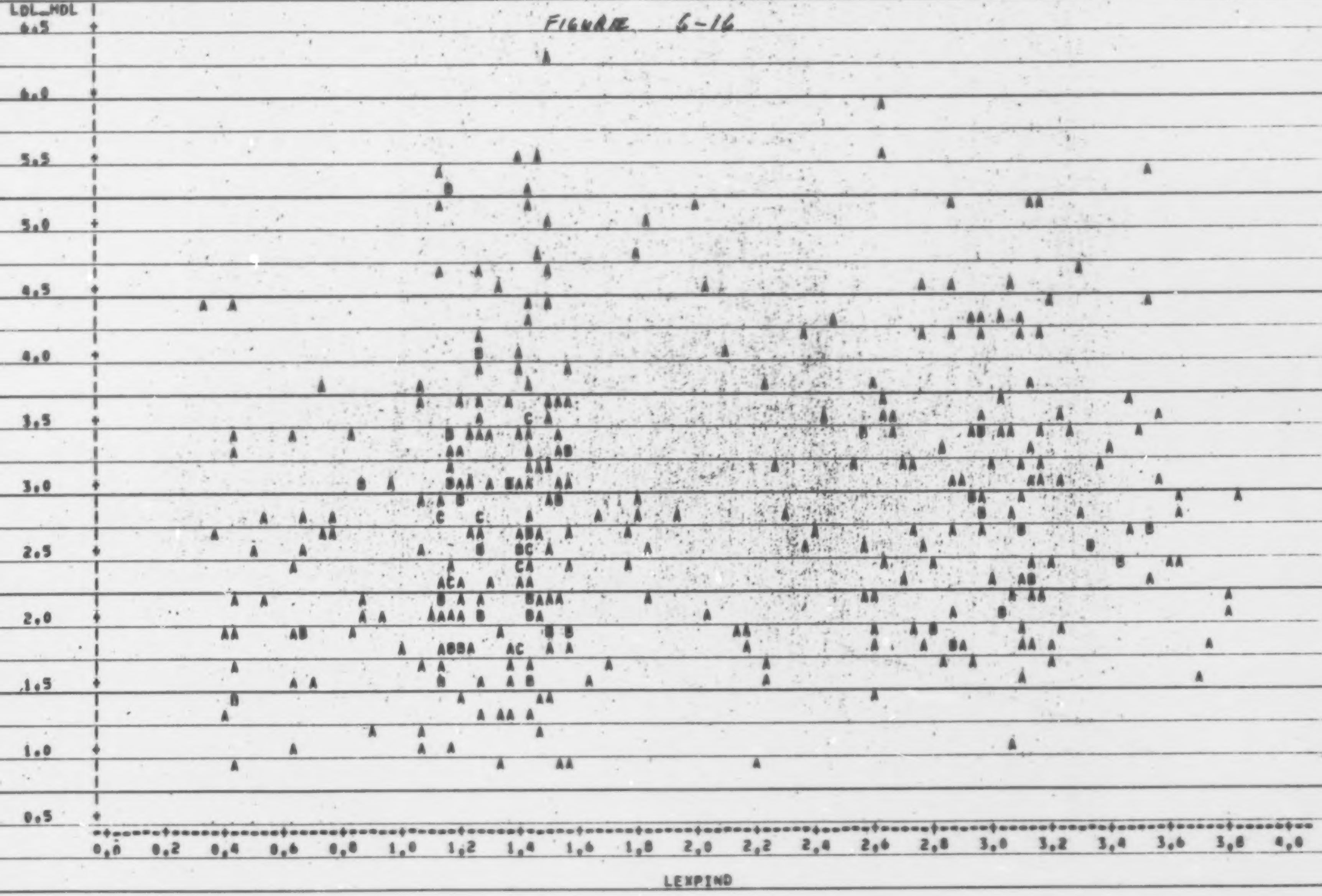
STATISTICAL ANALYSIS SYSTEM 16:35 TUESDAY, OCTOBER 20, 1981 5

PLOT OF LDL_MDL*LEXPIND LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 6-16



NOTE: 32 OBS HAD MISSING VALUES

CHAPTER 7 - EFFECTS OF CARBON DISULFIDE ON THE OCULAR SYSTEM

A. RETINAL PATHOLOGY

1. Literature Review

Retinal angiopathy was first associated with carbon disulfide when Goto and Hotta found increased incidence of retinal small hemorrhages or microaneurysms, nephropathy, anemia, and a variety of subjective symptoms among workers exposed to carbon disulfide in a rayon plant in Japan.[3,6] Direct ophthalmoscopy and fundus photography were used to study carbon disulfide exposed (CS_2 levels not reported) and comparison workers[6]. Direct ophthalmoscopy revealed that 44 of 338 exposed workers (13 percent) had retinal microaneurysms and that only 1 of 121 (0.8 percent) comparison subjects had microaneurysms (p less than 0.001). Fundus photography showed that 60 of 757 exposed (7.9 percent) and 4 of 269 (1.5 percent) comparison workers with retinal microaneurysms (p less than 0.001). A correlation between the frequency of microaneurysms and length of exposure was noted. With less than 6 years of exposure to CS_2 no workers had microaneurysms; with 6-10 years, 7 percent were affected; with 10-15 years, 14 percent; and with greater than 15 years, 12 percent.

In 1971 Goto et al.[3] performed fluorescein angiography on 195 Japanese workers exposed to CS_2 and 39 controls. Retinal microaneurysms were found in 55.9 percent of exposed and in 15.4

percent of comparison subjects. The exposed group tended to have more severe disease than controls and the prevalence of microaneurysms increased with length of exposure. Direct ophthalmoscopy and color fundus photography were also used to classify severity of microaneurysmal disease; the correlation with the fluorescein study was rather poor, suggesting that a great deal of disease was missed by the non-invasive techniques. Goto and coworkers used prednisolone-glucose tolerance testing (P-GTT) on this population in an attempt to evaluate a diabetogenic effect of CS_2. Fasting blood glucose values for exposed and comparison groups were similar, but the one and two hour glucose samples were both significantly higher in the exposed group (both p less than 0.01). When length of exposure was taken into account, glucose levels were higher in those exposed for longer periods of time.

Goto et al.[7] studied Yugoslavian viscose rayon workers and controls in 1972 with fluorescein angiography and prednisolone-GTT. Again more CS_2 exposed workers were found to have retinal microaneurysms than controls (29.1 percent vs. 11.1 percent). The frequency of microaneurysms increased with duration of exposure. No significant differences were found between cohorts in relation to the GTT.[7,8]

Although no information on exposure level was provided in these early reports, and they were often flawed by small comparison groups, there seemed no strong reason to doubt a connection between carbon disulfide exposure and a microangiopathy mediated by abnormal glucose metabolism until Raitta reported results of her examinations of 100 workers exposed to carbon disulfide in a Finnish rayon plant previously described by scientists at the Institute of Occupational Health in Helsinki.[5] Using oculosphygmography (ocular pulse wave), fluorescein angiography, and electrocardiogram to measure ocular hemodynamic alterations in 38 Finnish workers exposed to CS_2 (10-40 ppm) and 40 controls, they found significantly delayed peripapillary filling (p less than 0.01) in exposed workers compared to controls. The mean widths of 8 arterioles and the narrowest vein were significantly greater in the CS_2 exposed group (p less than 0.01 and p less than 0.05, respectively). Aneurysms were much less common in both the exposed and unexposed groups in Finland than in Japan; no effect of carbon disulfide was found on the prevalence of aneurysms; and there was no effect on blood sugar which could be detected by a standard glucose tolerance test not augmented with corticosteroids. Bias in selection of the Japanese cohort was considered the most likely explanation.

In 1976, Sugimoto, Goto and Hotta reported "a five-year follow-up on retinopathy due to carbon disulfide."[4] They concluded that retinopathy progressed more in the group that remained exposed and that regression in grade of retinopathy occured significantly more often in workers removed from carbon disulfide exposure than in those remaining in the same work site. While environmental levels again were not given, they recommended reduction in the Japanese TLV (60 mg/m^3, or about 19 ppm) for carbon disulfide.

Hotta et al.[8] defined by exposure a study group of 289 Japanese viscose rayon workers (124 high exposure, 127 low exposure and 38 mixed exposure) and 49 controls. In this instance, workers were studied with direct ophthalmoscopy and those with abnormal findings had their fundus color photographed. Retinal microaneurysms were then graded according to severity. The prevalence of retinal abnormalities (including microaneurysms, hemorrhages, and exudates) increased with both increased duration and exposure to CS_2.

In 1977, a cooperative study between Japanese and Finnish researchers was published.[9] The Japanese cohort consisted of 419 CS_2 exposed workers and 391 controls; the Finnish cohort of 188 CS_2 exposed, 76 controls. Environmental levels in the Japanese plant, described in 1976 by Tolonen and others, were in the same range as those in the Finnish plant.[2] Mean duration of exposure was 17 years for the Japanese and 14.2 years for the Finnish workers. Color fundus photography was used and Japanese and Finnish ophthalmologists analyzed the photos for "small red dots (microaneurysms and/or haemorrhages)". Twenty-four percent of the Japanese exposed workers had red dots and the prevalence increased with duration of exposure; only 3.7 percent of the Finnish exposed subjects had positive findings. The Japanese and Finnish comparison groups had 3.8 percent and 2.6 percent positive findings respectively. The ophthalmologic report essentially confirmed the earlier findings that retinopathy was rare in Finland, more common in Japan, and associated with carbon disulfide exposure in Japan but not in Finland.[9]

2. Methods

Each subject underwent pupillary dilation with a short acting mydriatic. After dilation was complete, direct ophthalmoscopy was performed and the results recorded. Each subject then had two pictures taken of each optic fundus using a Topcon retinal camera, a monochromatic light source, and panchromatic film. The film was later processed and mounted as black and white slides, identified only by the randomly-assigned study nmumber. The slides were initially read by two ophthalmologists, working independently, and each eye was reported as showing or not showing microaneurysms, as showing or not showing hemorrhages, and as showing or not showing other abnormalities. When we realized that this coding scheme was inadequate (see below), the slides were read by a third ophthalmologist and were reported separately for each eye as either normal, having definite or uncertain microaneurysms, or having definite or uncertain hemorrhages. As in the original readings, no information on exposure status was given to the reader, nor was he informed of the individual results of the first reading.

3. Results

a. From Current Exposure

In the first reading, both exposed and comparison groups were found to have a prevalence of microaneurysms of about 25%. Although our technique differed from those used in the Finnish and Japanese reports cited earlier, the high prevalence of microaneurysms in the comparison group was unexpected, even if an association of microaneurysms with carbon disulfide exposure exists. Since the concordance between the two independent readers was very good, (see table 7-1) it seemed possible that the population chosen for the study was, for some reason, unusual. Further inquiry and consultation with additional ophthalmologists revealed that the initial classification system had been too simple; experts advised that a large fraction of the slides could be expected to show small dots that were not clearly aneurysms but were also not clearly artifact. Arrangements were then made to have the slides read by a new ophthalmologist, as described above.

Table 7-2 shows results for the comparison versus exposed analysis, using the second grading method. The exposed group had almost 20 percent retinal microaneurysms (both definite and uncertain) compared to 7.5 percent for the comparison group (significant at p less than 0.01). The exposed group had 10.5 percent retinal hemorrhages (both definite and uncertain) compared to 3 percent for the comparison group (significant at p less than 0.01).

For statistical purposes, each job was placed into categories of definitely low exposure (DL), moderate exposure (M), or definitely high exposure (DH). The DL group was found to be exposed to less than 3 ppm; the M group to 3 ppm through 7.1 ppm; and the DH group to greater than 7.1 ppm. Analysis of prevalence by exposure level, as measured by NIOSH industrial hygeinists on two occasions in 1979, shows more retinal microaneurysms and hemorrhages in the exposed group than in the comparison group at $p = 0.04$. (See Table 7-2) There is an apparent increase in incidence of definite plus uncertain microaneurysms as exposure to carbon disulfide increases from low to medium to high. No such trend is apparent for hemorrhages nor for definite aneurysms alone.

b. From Cumulative Exposure

Smoking histories differed between exposed and comparison groups. Since cigarette smoking might reasonably be expected to correlate with vascular disease, pack-years were used as an independent variable in analysing the cumulative effect of CS_2 exposure. Table 7-3 shows the results of a logistic regression of microaneurysms and hemorrhages on age, pack-years, log(exposure index), and the interaction of age and log(exposure index), where the exposure index (exp. ind.) for a worker is the product of the historical CS_2 level in each job in the plant for a given year and the number of months in that year the worker spent in the job. Log(exp. ind.) showed an association with retinal microaneurysms only when definite and uncertain readings were grouped together. Statistically significant associations were found between hemorrhages and log(exp. ind.) irrespective of whether the "uncertain" readings were excluded, counted as normal, or counted as abnormal.

4. Discussion

Fluorescein angiography is by far the best technique for absolute determination of the type of pathology thought to be associated with chronic carbon disulfide exposure (i.e. microaneurysms and/or small point hemorrhages). This information was known to Sugimoto et al.[9] when they decided to use only color fundus photography for their study and to record the presence or absence of "small red dots" which comprised both microaneurysms and hemorrhages. They were satisfied that their accuracy of detection of relative retinal abnormalities was as good as either fluorescein angiography or the more elaborate systems of grading used in Sugimoto's 1976 study.

Time constraints and safety concerns in the field prohibited use of fluorescein angiography in this survey of 446 subjects, despite the evidence to suggest that it produces the best sensitivity and specificity. Retinal photography with monochromatic light source was chosen based on information from Delori and Gragoudas[10] that claimed "excellent demonstration of the retinal vasculature by monochromatic light at 570 nm". The method used to report findings from the retinal slides depends on the method of photography: whereas Sugimoto's study[9] made decisions of severity based on the presence of "small red dots", this study made decisions of definite or uncertain microaneurysms and hemorrhages based on the presence of "small black dots."

The statistical analysis comparing controls to all workers exposed to carbon disulfide shows that the exposed subjects have a statistically significant increase in microaneurysms (p less than 0.01). The relationship between hemorrhages and CS_2 exposure is stronger (p less than 0.01) and holds true for cumulative exposure as well as for current exposure. These data seem to confirm the experience of the Japanese researchers who found increased incidence of retinal microaneurysms in workers exposed to CS_2 and contrasts with the experience of the Finnish researchers, who found no increase. The actual proportions are about half as great as those reported in Japan but about equal to those found in Yugoslavia.

The absence of a dose-response correlation calls for caution in interpreting the findings as due to CS_2 exposure. Just as Sugimoto et al. were left with no explanation for the differences between Japanese and Finnish workers, we can offer no new conjecture explaining the difference in response between American, Japanese, and Finnish workers.

The wider biological significance of retinal changes is also not as clear as one would wish. Their presence is particularly troubling when seen in the light of other studies showing an excess of cardiovascular deaths[1] in carbon disulfide workers, of some studies showing significant renal vascular disease, and of other weak but statistically significant correlations between exposure and cardiovascular risk factors found in this study.

B. REFERENCES

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C. SUMMARY

To determine the effect of CS_2 exposure on retinal vasculature, each subject in the study underwent pupillary dilation with a short acting mydriatic, direct ophthalmoscopy, and retinal photography with monochromatic light. The photographs were read by an ophthalmologist, who was not aware of the identity of the subjects whose slides he was evaluating, and rated as normal, having definite or uncertain microaneurysms, or having definite or uncertain hemorrhages. The exposed group had almost 20 percent retinal microaneurysms (both definite and uncertain) compared to 7.5 percent for the comparison group (significant at p less than 0.01). The exposed group had 10.5 percent retinal hemorrhages (both definite and uncertain) compared to 3 percent for the comparison group (significant at p less than 0.01). There was an apparent increase in incidence of definite plus uncertain microaneurysms (combined) as exposure to carbon disulfide increases from low to medium to high. No such trend was apparent for hemorrhages nor for definite aneurysms alone. Log(exp. ind.) showed an association of cumulative exposure to CS_2 with retinal microaneurysms when definite and uncertain readings were grouped together. Statistically significant associations were found between hemorrhages and log(exp. ind.) irrespective of whether the "uncertain" readings were excluded, counted as normal, or counted as abnormal. These findings are consistent with others showing an association between CS_2 exposure and small artery disease.

Table 7-1
Concordance in Interpretation of Retinal Photographs by Initial Two Readers - Microaneurysms

		Reader "M"				
			Microaneurysms Present			
		Normal	Right	Left	Both	Total
Reader "R"	Normal	328	1	1	5	335
Microaneurysms Present:	Right	0	6	0	1	7
	Left	7	0	3	1	11
	Both	7	3	6	79	95
	Total	342	10	10	86	448

Table 7-2: Prevalence of Retinopathy by Exposure Level
Number (Percent)

Microaneurysms

	Definite	Uncertain	Abnormal*	Normal	Total
Comparison	11 (4.8)	6 (2.6)	17 (7.5)	211 (92.5)	228
Low	6 (13.3)	2 (4.4)	8 (17.8)	37 (82.2)	45
Medium	8 (12.5)	4 (6.3)	12 (18.8)	52 (81.2)	64
High	7 (15.9)	3 (6.8)	10 (22.7)	34 (77.3)	44
χ^2 (d.f.)	13.44 (6)		13.08(3)		
P value	0.04		LT 0.01		
All Exposed	21 (13.73)	9 (5.88)	30 (19.61)	123 (80.39)	153
χ^2 (d.f.)	12.64 (2)		12.50(1)		
P value	LT 0.01		LT 0.01		
Total	32 (8.4)	15 (3.9)	47 (12.3)	334 (87.7)	381

*Abnormal = Definite + Uncertain

Hemorrhages

	Definite	Uncertain	Abnormal*	Normal	Total
Comparison	6 (2.63)	1 (0.44)	7 (3.1)	221 (96.93)	228
Low	5 (11.11)	0 (0.00)	5 (11.1)	40 (88.89)	45
Medium	7 (10.94)	0 (0.00)	7 (10.9)	57 (89.06)	64
High	3 (6.82)	1 (2.27)	4 (9.1)	40 (90.91)	44
χ^2 (d.f.)	13.26 (6)		9.01(3)		
P value	0.04		0.03		
All Exposed	15 (9.80)	1 (0.65)	16 (10.46)	137 (89.54)	153
χ^2 (d.f.)	9.16 (2)		8.81(1)		
P value	0.01		0.01		
Total	21 (5.51)	2 (0.52)	23 (6.0)	358 (93.96)	381

*Abnormal = Definite + Uncertain

Table 7-3: Results of Multiple Logistic Regression of Retinal Microaneurysms and Hemorrhages on Age, Cigarette Pack Years, Log(Exposure Index), and Age.Log(Exposure Index)

	Excluding Uncertain		Abnormal[1]		Uncertain Counted as Normal	
	Q2	beta[3]	Q2	beta[3]	Q2	beta[3]
Microaneurysms:						
Intercept		-4.88*		-4.29*		-4.99*
QAge, Pack-Years, LExp, Age.LExp	8.79		20.26*		7.57	
QAge	5.12*	0.042	10.61*	0.020	4.40*	0.049
QPack-Years	0.18	$7.2\text{x}10^{-5}$	0.62	$4.33\text{x}10^{-5}$	0.40	$-1.20\text{x}10^{-5}$
QLExp	3.40	0.663	9.02*	0.637	2.59	0.693
QAge.LExp	0.09	-0.006	0.01	-0.001	0.18	-0.008
Hemorrhages:						
Intercept		-11.13*		-8.21*		-11.15*
QAge, Pack-Years, LExp, Age.LExp	24.85*		21.99*		24.72*	
QAge	14.04*	0.162*	12.89*	0.096	14.01*	0.163
QPack-Years	0.29	$1.5\text{x}10^{-7}$	0.15	$7.0\text{x}10^{-6}$	0.32	$2.20\text{x}10^{-6}$
QLExp	8.08*	2.536*	8.27*	1.570	7.91*	2.539*
QAge.LExp	2.44	-0.041	0.68	-0.020	2.48	-0.041

[1] Abnormal = Definite + Uncertain; [2] Q = Q Statistics; [3] beta = regression coefficient for full model; LExp = Log(Exposure Index)
*Significant at p less than 0.05.

CHAPTER 8 - EFFECTS OF CARBON DISULFIDE ON THE NERVOUS SYSTEM

A. INTRODUCTION:

The neurotoxic effects of carbon disulfide on the peripheral nervous system are well known[12]. Studies on viscose rayon workers have shown that CS_2 exerts a general neurotoxic effect, including peripheral nervous system (PNS) toxicity. The PNS effects include reduction in motor conduction velocity;[2,6,14,15,16,17] sensory conduction velocity[17]; lengthened motor and sensory distal latencies[14]; tremor[6]; and abnormal electromyograms[2]. A common feature of all these epidemiological studies is that workers' CS_2 exposures had exceeded 20 ppm[12], the current U. S. occupational Permissible Exposure Limit, sometime during their employment.

In addition to epidemiological studies on workers, investigations with laboratory animal have shown that CS_2 reduces nerve conduction velocities[7,9,13] and a recent review[12] states that "experimental morphological studies show that subchronic CS_2 intoxication manifests itself essentially as an axonal degeneration."

There is therefore considerable evidence from epidemiological studies on viscose rayon workers, as well as from experimental animal data, that CS_2 adversely affects PNS function. What has not been reported in earlier studies is whether CS_2 exposure at levels equal to or lower than 20 ppm is deleterious to the PNS.

B. METHODS AND MATERIALS:

Details of the plant environment are described in Chapter 2; the composition of the exposed and control groups is reviewed in Chapter 5; behavioral and CNS effects are described in Chapter 9 and by Putz-Anderson et al.[10]

Our evaluation of each subject's peripheral nervous system was based on two types of data. Electrodiagnostic tests were administered to obtain objective data bearing on the functional status of selected peripheral nerves. In addition, we analyzed workers' responses from the medical history questionnaire to assess the prevalence of symptoms related to PNS impairment.

1. Data Collection

The electrodiagnostic tests consisted of nerve conduction velocity measurements on the ulnar, peroneal, and sural nerves. Standard methodology, using surface electrodes, was employed[3,4]. Maximum motor conduction velocity (MCV) was measured in the ulnar and peroneal nerves; sensory conduction velocity (SCV) was measured in the sural nerve. Ulnar MCV was measured between elbow and wrist; peroneal MCV between knee and ankle. For the sural nerve, orthodromic stimulation was used, with the stimulus site slightly distal and lateral to the malleolus. As part of the motor conduction procedure, we recorded both the distal latency and amplitude (peak to peak) of compound muscle action potentials. As part of the sensory conduction velocity test, we recorded the latency to the first negative peak of the sural nerve action potential (NAP) and the peak to peak amplitude of the NAP.

Two parameters pertaining to the ulnar and peroneal nerves were calculated from the MCV data: residual latency for each nerve and amplitude ratio for compound muscle action potentials (MAP). The amplitude ratio was calculated by dividing the peak-to-peak amplitude of the MAP recorded in response to stimulation at the proximal location on the nerve by the MAP's amplitude obtained from distal stimulation.

All testing was conducted at the company's site in unoccupied rooms adjacent to the employee health unit. Testing was conducted by ten occupational health graduate nursing students from the University of Cincinnati. Each student was given a minimum of ten hours of NCV training by a NIOSH investigator over a three week period prior to the beginning of the study. A NIOSH senior investigator (or an assistant) was always in attendance during the study to provide assistance and handle special problems when the need arose. All participants were received blind at the neurologic test station, i.e., neither the nurses nor the investigator (or assistant) knew any participant's exposure group identity. Participants were scheduled randomly within constraints of shift assignment; forms were coded so that the investigators did not know to which group a given subject belonged.

All conduction velocities were measured on supine subjects. Right extremities were tested unless the subject reported an injury or disease to those limbs; in such cases, the left extremity was tested. All subjects reported prior to their day's work; therefore no CS_2 exposure had occurred for at least 8 hours prior to the test. Since all subjects were present at the examination site for 30 to 120 minutes prior to testing, equilibrium with room temperature had occurred by the time of testing. Room temperature was uncontrolled and varied from the upper 60's to the mid 80's (Fahrenheit) during the test sessions. Test sessions were conducted during both daytime and nighttime; however approximately 75% of the participants were tested during daytime sessions. At times, when excessive scheduling delays occurred at the testing station, the ulnar nerve observation was eliminated from the test battery; as a result, only approximately 60% of the study participants had ulnar MCV measurements.

In order to correct for the effect of nerve temperature on conduction velocity, skin surface temperatures were measured for each subject. Temperature measurements for the ulnar and peroneal nerves were recorded midway between the stimulus sites (following the natural nerve pathway) and midway between the stimulation and recording electrodes for the sural nerve. From these data, a mode temperature across all subjects was calculated for each nerve. This mode temperature was entered into deJesus et al.'s[1] formula in order to adjust each worker's conduction velocity data to a common temperature. We also used a similar procedure to adjust distal latencies to both a common temperature as well as to a common distance between stimulation and recording electrodes. (See Appendix.)

The medical questionnaires completed by all study participants contained 12 questions related to neurological symptoms (see Table 8-1). Seven of the questions were specific to the central nervous system; the remaining five questions related to both CNS and PNS symptoms.

2. Statistical Methods

In order to assess the dose/effect relationship between CS_2 exposure and peripheral nerve data, participants were classified into either exposed or comparison groups with further subclassification of the exposed group into definitely high (DH), moderate (M), and definitely low (DL) categories, based on each person's current job assignment and the assessment of the exposure potential within that job assignment which was made by NIOSH and company industrial hygienists before the start of data collection. The DL group was found to have current exposures of approximately 0-3 ppm; the M group 3-7.1 ppm; and the DH group, greater than 7.1 ppm.

Group differences for the neurologic data were assessed using multivariate analysis of variance techniques. The comparison group was compared to the DH, M, and DL, groups taken together and to the individual exposure levels, i.e. DH, M, and DL, separately. Age was found to be an acceptable covariate. Therefore, except for the residual latency data, which had been found to be uncorrelated with age, the testing of group differences was performed using analysis of covariance (ANCOVA) to adjust for the confounding effects of age.

We also examined the data for significance between groups by combining neurophysiological data for each nerve, then testing differences between groups using multivariate analysis of covariance (MANCOVA). Distal latency, amplitude ratio (discrete amplitude for sural), and nerve conduction velocities were used as the dependent variables; the independent variable was group, with age as a covariate. The Hoetelling-Lawley trace was used to determine significant differences. Univariate analysis were included to assess the contribution of individual variables included in the multivariate analyses (Ramsey, 1980).

Two statistical methods were used to explore within the combined CS_2-exposed groups the effects of long-term exposure: we first looked at the association between each neurophysiological variable and the following variables: age, length of employment, and cumulative exposure index. The cumulative exposure index for each worker was calculated by summing, over the worker's total employment, the product of the CS_2 level (ppm) and months worked at that level (See Chapter 3). The multiple regression technique was employed, using combinations of variables and selected order of entry of the variables into the model.

In the second method, a two-way analysis of variance was used to evaluate dose-effect relationships for the peroneal and sural conduction velocities. Study participants were grouped according to level of cumulative exposure index and blocked length of exposure. The levels were arbitrarily chosen. After the deletion of 14 participants who had begun work at the rayon staple plant after age 40, a high correlation between worker's age and length of employment was found; therefore, controlling for length of employment essentially also controlled for age.

Workers' reporting of symptoms related to PNS disorders were analyzed using a chi-square test. The differences in response rates between the total CS_2-exposed group and the comparison group and between the three CS_2 groups and the comparison group were evaluated for each symptom. This procedure was followed for single and multiple reporting of symptoms alike.

Of the 233 eligible participants in the comparison group and 156 eligible participants in the exposed group, the number tested varied, depending on the nerve being tested. As stated above, time constraints prevented testing all workers for each of the three nerves. Additionally, some workers' data were excluded if indications of diabetes, excessive alcohol consumption, or exposure to lead were present. Each of these conditions can produce peripheral neuropathy, thus potentially confounding the data. Specifically, a participant was excluded from analysis if one or more of the following occurred:

1) Answered positively to the questionnaire item "Are you taking medicine for high blood sugar or sugar in the urine?"
2) Answered positively to the questionnaire item "Have you been told by a doctor you have sugar diabetes?"
3) Reported having consumed 35 or more drinks (sum of beer, wine, cocktails, liqueurs, and bootleg corn liquor) during the week prior to the study.
4) Had fasting blood sugar value greater than 110 mg/dl; or
5) Had blood lead value of 40 mcg/dl or more.

The resulting study groups did not differ in terms of blood lead levels or alcohol consumption.

These exclusion criteria caused a decrease in total number of workers within the exposed and comparison groups from 156 and 233 to 145 and 212, respectively. Table 8-2 contains the specifics of the main study groups; the mean age of the CS_2-exposed group was 4.6 years greater than that of the comparison group.

Residual latency (time for impulse transmission across the motor end plate and depolarization of muscle fibers) was determined to assess dysfunction in neuromuscular junctions. The procedures used for data adjustments and calculations are provided in the Appendix.

C. RESULTS

1. Neurologic Measures

The motor conduction velocity for the comparison group (Table 8-3) was in close agreement with the published mean normal value, 57.5 M/S, for the ulnar nerve[3]. Our sural SCV and peroneal MCV results for the comparison group, however, are somewhat lower than published mean normal values of 46.2 M/S and 50.0 M/S, respectively[3]. For both nerves, our results are about 10 percent lower, but both would be considered within the normal range for these two nerves.

Simple descriptive statistics (mean, standard deviation) are given in Tables 8-3a and 8-3b for the neurophysiologic test measures, adjusted to standard experimental reference conditions (temperature and terminal distance). Also, corresponding means adjusted for the subject's age are provided, except for residual latency where a nonsignificant correlation with age was determined, as previously mentioned.

Table 8-3b contains the results from the group comparisons of the neurophysiological data shown in Table 8-3a. The results are summarized for each nerve tested and indicate the following:

a. Ulnar Nerve - No group differences were found for the three variables (distal latency, amplitude ratio, motor conduction velocity) relating to the ulnar nerve. The results of the regression analysis indicated that age or employment length explained a small (R^2 LTE 0.11), but significant (p LT 0.05) portion of the variability in amplitude and conduction velocity measurements. Including the CS_2 cumulative exposure index as an independent variable did not significantly improve the model. None of the exposed groups had MCVs differing significantly from the mean of the comparison group (56.7 m/s, Table 8.3a).

b. Peroneal Nerve - The MANCOVA results indicated significant group differences for peroneal nerve measurements (Table 8-4); the univariate results suggest that the differences occurred for both the amplitude ratio and motor conduction velocity (Tables 8-3a and 8-3b). The sub-group having the highest current CS_2 exposure had the greatest decrease in both amplitude and velocity (Table 8-3b).

The regression analyses of the exposure group data indicated that either age or employment length are significant factors (R^2 GTE 0.10) in explaining MCV variability. (These results were not found for distal latency or amplitude ratio). Furthermore, when the cumulative exposure index was introduced in the model for peroneal MCV, a statistically significant increase in explained variability (R^2 increased from 0.19 to 0.21) was found when age was the previous factor introduced in the model. The factor employment length became nonsignificant when cumulative exposure index was introduced.

Stratification of peroneal MCV results by cumulative exposure index and length of employment (Table 8-5) indicates that increases in the cumulative exposure index were associated with a reduction in the peroneal MCV (p LT .05). "Length of employment" was not significantly associated with decreased peroneal MCV.

Comparison of differences in frequency of "no observed action potential" showed no significant differences between groups (p GT 0.05) for the peroneal nerve. (Table 8-6)

c. Sural Nerve - A significant decrease in the sural nerve sensory conduction velocity was noted in the exposed workers, as compared to the unexposed (Tables 8-3a and 8-3b). The regression analyses indicated that neither age nor length of employment was a significant variable in the model; cumulative exposure index was also not significant when entered as the second factor. The ANOVA results were similar to those for peroneal conduction velocity - length of employment was non-significant, cumulative exposure was. However, the results were confounded by a significant interaction between the variables "length of employment" and "cumulative exposure index". Put another way, inconsistencies within the exposure groups permit no direct interpretion of the association between sural SCV and the cumulative exposure index.

Comparison of differences in frequency of "no observed action potential" showed no significant differences (p GT 0.05) for the sural nerve for the total exposed group versus the comparison group (Table 8-6). However, a significant difference (p LT 0.02) was found for the sural nerve action potential when the three exposure groups were analyzed separately. There was therefore suggestive evidence that increased CS_2 exposure levels raised the sural nerve's threshold for stimulation.

2. Symptoms

The number and prevalence of reported symptoms compatible with PNS disorders are given in Table 8-7. No significant differences were reported for any symptom between the CS_2-exposed group and the comparison group. Furthermore, no significant differences in reporting PNS symptoms were found when the three CS_2 subgroups were individually compared to the comparison group.

D. DISCUSSION

The results from this investigation demonstrate a reduction in nerve conduction velocities associated with chronic, low level exposure to CS_2. The reductions were confined to the peroneal and sural nerves; no effect on the ulnar nerve was found. The reduction in sural SCV in the rayon staple group was possibly associated with an apparent reduction in excitability of the nerve. The frequency of "nonresponders," i.e. those subjects from whom we were unable to elicit a sural nerve action potential, increased across the CS_2-exposed groups. For the peroneal nerve, the reduction in MCV was greater than for the sural SCV. Associated with the reduced peroneal MCV was a decrease in the ratio of the amplitude of muscle action potentials. It appeared that the amplitude of muscle action potentials elicited by stimulation at the knee of the peroneal nerve was reduced. This decrease in amplitude could be due to a slowing in some nerve fibers, causing flattening of the muscle action potential. For both nerves, the slightly reduced conduction velocities, together with small decreases in muscle or nerve action potentials present a picture of mild axonopathy. This possibility would be consistent with data from experimental animal studies[12].

A dose-effect relationship was found between CS_2 exposure and the peroneal MCV data. The greater the CS_2 concentration-time index, the greater the MCV decrement. Length of employment was not a significant factor. This latter finding may be due to compensatory recovery mechanisms that prevent any further degradation of peroneal MCV with additional years of low CS_2 exposure. For example, Seppalainen and Linnoila[9] reported partial recovery of MCV in rats exposed to CS_2, then followed for improvement. Similar recovery mechanisms could be present in the human population surveyed in this study.

The results from this investigation are in partial agreement with those from previous studies of viscose rayon workers[6,14,17]. Our finding of reduced MCV in the peroneal nerve agrees with Seppalainen and Tolonen's results[14]; however, we did not obtain similar findings for the ulnar nerve. The Finnish viscose rayon workers apparently had experienced higher CS_2 exposures than our sample of rayon workers. The investigators reported that CS_2 exposure had at times reached 60 ppm, but not exceeded 30 ppm for the 12 years preceding their study. Our workers, in contrast, apparently had not generally been exposed to CS_2 levels exceeding 20 ppm, since company records showed the plant to have always been in compliance with the U. S. permissible exposure level for CS_2. Our finding that sural SCV was reduced in rayon workers is in agreement with Vasilescu[17], who, however, did not find reductions in the MCV's of the median, peroneal, and ulnar nerves. Vasilescu concluded from his study that sensory nerve fibers show the earliest signs of subclinical polyneuropathy in CS_2 exposures, although his study contained no exposure data. Our results do not support a similar conclusion. Reductions in sural SCV and peroneal MCV were both found to be significant in our investigation. Indeed, it is difficult to explain selective toxicity on the basis of sensory versus motor function.

Seppalainen and Haltia[12] note that CS_2-induced neuropathy is usually accentuated in the legs. Our results are in agreement in that the ulnar nerve differences were not significant, whereas the sural and peroneal data were significantly different from the comparison group's data.

We did not find a significant increased reporting of PNS symptoms in CS_2-exposed workers. This lack of finding is in distinction to the reports of Lilis[8], Knave et al.[6], Rizzo and Franco[11], and Vanhoorne[16]. In each of these references the authors reported an increased prevalence of symptoms of polyneuritis or polyneuropathy. The frequency and type of symptoms, however, decreased with reduced CS_2 exposure levels. For example, Lilis's subjects were workers presenting signs of CS_2 poisoning; prevalence of symptoms of peripheral neuropathy was high. Rizzo and Franco reported symptoms of polyneuritis, but only in workers with chronic exposure at CS_2 levels exceeding 30 mg/m^3 (10 ppm). The CS_2 exposure levels in our study were lower than those in the aforementioned investigations. It is therefore suggested that symptoms of peripheral nerve polyneuritis are not manifested at CS_2 exposure levels below 10 ppm.

The apparent association found in this study between reduced nerve conduction velocities and low-level CS_2 exposure requires placing in perspective as concerns individual workers' health. It should be borne in mind that the reductions in nerve conduction velocities (NCV) were still within a range of clinically normal values. Indeed, the decrements noted were relatively small departures from the comparison group's mean values. One must therefore ask as to the health significance of the results. Without further evidence to the contrary, it is difficult to postulate specific health consequences with reductions in NCV's of the magnitude reported here. The data indicate, in the opinion of the authors, that minimal neurotoxicity is evident. We cannot associate specific health consequences with the reductions in NCV reported here, but these results do point out the continuing need for industrial hygiene practices designed to minimize workers' exposure to carbon disulfide.

E. REFERENCES

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F. SUMMARY

The effects of occupational exposure to carbon disulfide were examined in a sample of 156 male viscose rayon workers; a composite group of 233 workers drawn from two other artificial fiber production plants located on the same premises as the rayon plant, but not using carbon disulfide, was used for comparison. Effects of CS_2 on the peripheral nervous system (PNS) were evaluated in the ulnar and peroneal nerves using measurements of motor nerve conduction (MCV) velocity and distal latency; sensory conduction velocity (SCV) was evaluated in the sural nerve. Self-reported symptoms of PNS disorders were obtained from each study participant. Industrial hygiene records showed the rayon workers had CS_2 exposures that had generally not exceeded 20 ppm. The comparison group's mean CS_2 level approximated 0.2 ppm. Results showed CS_2-exposed workers to have statistically significant reductions in sural SCV and peroneal MCV. Other neurologic motor nerve computed variables consisting of distal latency, residual latency, and action potential amplitude ratio showed no statistical significant differences except for reduction in amplitude ratio for the peroneal nerve ($P = 0.05$). Reductions in the peroneal nerve MCV were found to be related, in a dose-response sense, to workers' cumulative exposure to CS_2. No increase attributable to CS_2 was found in the prevalence of symptoms related to PNS disorders. The results from this study generally agree with similar findings from Finland, Sweden, and Italy, but at CS_2 exposure levels lower than those previously reported for occupational exposure.

G. APPENDIX - DATA ADJUSTMENT METHODS

The NCV data were adusted to compensate for differences in subjects' temperature and terminal distances. To correct for temperature effects, a mode temperature was calculated for each limb across all participants in the study. The following data adjustments were then performed:

1. Ulnar or Peroneal Motor

a. NCV's were corrected to the mode of the midpoint nerve temper-atures using DeJesus *et al*.'s[1] temperature correction equation below.

$V_s = V_E e^{0.0419\Delta T}$ meters/second, where V_s = corrected (or standardized) velocity to a standard (mode) temperature,

V_E = measured velocity at the experimental temperature, and

ΔT = difference in °C between the standard (mode) and experimental temperatures.

b. Distal Latencies were corrected to the mode of distal temperatures using DeJesus' temperature correction equation below[1].

$DISL_s = DISL_E e^{-0.0507\Delta T}$ milliseconds, where $DISL_s$ = corrected (or standardized) distal latency to a standard (mode) temperature,

$DISL_E$ = measured distal latency at the experimental temperature, and

ΔT = difference in °C between the standard (mode) and experimental temperatures.

c. Residual latencies were calculated by subtracting from the standardized distal latency (derived in b.) the terminal distance divided by the standardized NCV (derived in b.).

$RL = DISL_s = {}^{S}/_{V_s}$ milliseconds, where RL is residual latency, $DISL_s$ is standardized distal latency, S is terminal distance, and V_s is velocity standardized to the distal mode temperature.

d. Distal latencies (derived in b.) were corrected to the mode of the nerve terminal distances using the equation below.

$L_s = DISL_s + {}^{\Delta S}/_{V_s}$ milliseconds, where $DISL_s$ = latency corrected (or standardized) to a standard (mode) distance, L_s = latency standardized to the distal mode temperature, V_s = standardized velocity to the distal mode temperature, and ΔS = difference between the standard (mode) and experimental terminal distances.

e. Ratio of amplitudes (proximal MAP divided by distal MAP) was calculated.

2. Sural Sensory

The only standardizations performed on the sural nerve consisted of correcting the velocities to the mode of the midpoint nerve temperatures using the equation in 1a. above.

Table 8-1: Neurological History:
Symptom Identification (ID), Description, and Affected

Symptom ID	Description	Affected System
1	Muscle weakness (paresis)	PNS or CNS
2	Blurring vision	CNS
3	Trembling or shaking hands	PNS or CNS
4	Difficulty in remembering things	CNS
5	Difficulty in walking	PNS or CNS
6	Dizziness	CNS
7	Difficulty in getting to sleep	CNS
8	Frequently felt tired	CNS
9	Frequent headaches	CNS
10	Unexplained numbness or tingling in hands or feet	PNS or CNS
11	Treated for mental or psychiatric condition	CNS
12	Pain in either leg	PNS

PNS = peripheral nervous system

CNS = central nervous system

Table 8-2: Study Participants Excluded

		Comparison	Exposed	Total
Eligible for Inclusion		233	156	389
Tested, but Excluded from Data Analysis				
Diabetes		2	2	4
Drink	GTE 35 drinks	13	2	15
Fasting Glucose	GTE 110 mg/dl	3	3	6
Blood Lead	GTE 40 mcg/dl	3	4	7
Subtotal		21	11	32
Nonexcluded Receiving Tests		212	145	357

Peroneal

	Comparison	Exposed	Total
Data Collected	199	137	336
Data Not Collected	13	8	21

$\text{Chi-square}_1 = 0.06$; p greater than 0.80

Sural

	Comparison	Exposed	Total
Data Collected	198	130	328
Data Not Collected	14	15	29

$\text{Chi-square}_1 = 1.61$; p greater than 0.20

Ulnar

	Comparison	Exposed	Total
Data Collected	105	85	190
Data Not Collected	107	60	167

$\text{Chi-square}_1 = 2.86$; p between 0.05 and 0.10

	Comparison	Exposed
Mean Age (± S.D.)	33.9 (9.0)	38.5 (10.1)

Table 8-3a: Summary Statistics ($\overline{X}$, SD) for Standardized Neurologic Test Results

Variable	Units	Comparison Group				Exposed Group			
		N	$\overline{x}$	$\overline{x}^a$	SD	N	$\overline{x}$	$\overline{x}^a$	SD
Ulnar Motor									
Distal Latency	ms	101	3.3	3.3	0.7	85	3.3	3.3	0.9
Residual Latency	ms	101	2.0		0.7	85	2.09		0.9
Amplitude Ratio	None	102	1.0	1.0	0.3	83	0.9	1.0	0.2
MCV	m/s	104	56.9	56.7	6.75	85	55.9	56.2	6.29
Peroneal Motor									
Distal Latency	ms	193	4.0	4.0	0.8	135	4.1	4.1	0.8
Residual Latency	ms	193	2.8		0.8	135	2.9		0.8
Amplitude Ratio	None	198	0.9	0.9	0.2	137	0.8	0.9	0.2
MCV	m/s	196	45.3	45.0	4.4	136	43.2	43.5	4.9
Sural Sensory									
Latency	ms	198	4.2	4.2	0.7	130	4.3	4.3	0.8
Amplitude	mV	198	3.8	3.8	2.0	128	3.6	3.7	2.1
SCV	m/s	198	41.8	41.6	3.4	130	40.4	40.6	4.0

$\overline{x}_a$ = Age Adjusted Mean

[1] Skin temperature midway over nerve pathway between the proximal and distal stimulus sites.

Table 8-3b: Summary Statistics ($\overline{X}$, SD) for Standardized Neurologic Test Results

		Distal Latency ms	Residual Latency ms	Amplitude Ratio None	MCV m/s
Ulnar Motor					
Comparison Group	N	101	101	102	104
	$\bar{x}$	3.3	2.0	1.0	56.9
	$\bar{x}_a$	3.3		1.0	56.7
	SD	0.7	0.70	0.3	6.7
Exposed Group					
DL	N	26	26	26	26
	$\bar{x}$	3.2	1.8	1.0	55.5
	$\bar{x}_a$	3.2		1.0	55.5
	SD	0.4	0.4	0.2	6.4
M	N	35	35	34	35
	$\bar{x}$	3.4	2.1	1.0	56.8
	$\bar{x}_a$	3.4		1.0	57.2
	SD	1.1	1.1	0.1	6.0
DH	N	24	24	23	24
	$\bar{x}$	3.4	2.0	0.9	55.0
	$\bar{x}_a$	3.4		0.9	55.4
	SD	1.0	1.0	0.2	6.6
Peroneal Motor					
Comparison Group	N	193	193	198	196
	$\bar{x}$	4.0	2.8	0.9	45.3
	$\bar{x}_a$	4.0		0.9	45.0
	SD	0.8	0.8	0.2	4.4
Exposed Group					
DL	N	44	44	44	44
	$\bar{x}$	4.2	3.1	0.9	43.7
	$\bar{x}_a$	4.2		0.9	44.2
	SD	0.7	0.7	0.2	5.1
M	N	55	55	57	56
	$\bar{x}$	4.0	2.9	0.9	43.4
	$\bar{x}_a$	4.0		0.9	43.8
	SD	1.0	0.9	0.2	4.8
DH	N	36	36	36	36
	$\bar{x}$	3.9	2.7	0.8	41.8
	$\bar{x}_a$	4.0		0.8	42.3
	SD	0.6	0.7	0.2	4.5

Table 8-3b: Summary Statistics ($\bar{X}$, SD) for Standardized Neurologic Test Results (Cont'd)

		Latency ms	Amplitude None	SCV m/s
Sural Sensory				
Comparison Group	N	198	198	198
	$\bar{x}$	4.2	3.8	41.8
	$\bar{x}_a$	4.2	3.8	41.6
	SD	0.7	2.0	3.6
Exposed Group				
DL	N	39	38	39
	$\bar{x}$	4.2	3.2	41.2
	$\bar{x}_a$	4.2	3.3	41.3
	SD	0.9	1.7	5.1
M	N	60	60	60
	$\bar{x}$	4.4	3.4	39.8
	$\bar{x}_a$	4.4	3.5	40.1
	SD	0.8	1.5	3.7
DH	N	31	30	31
	$\bar{x}$	4.2	4.4	40.5
	$\bar{x}_a$	4.2	4.4	40.6
	SD	0.7	3.2	3.0

$\bar{x}_a$ = Age Adjusted Mean

[1] Skin temperature midway over nerve pathway between the proximal and distal stimulus sites.

Table 8-4
MANCOVA and Univariate Results of Neurological Measurement Comparisons

Variable	Exposed vs. Comparison F (df)	P	DL vs. M vs. DH F (df)	P
Ulnar Motor				
*Latency, Amp. Ratio, MCV	0.53 (3,177)	0.67	0.56 (9,521)	0.83
Distal Latency	0.22 (1,183)	0.64	0.48 (3,181)	0.70
Amplitude Ratio	1.60 (1,182)	0.21	0.71 (3,180)	0.55
MCV	0.27 (1,186)	0.60	0.59 (3,184)	0.62
Peroneal Motor				
*Latency, Amp. Ratio, MCV	4.78 (3,322)	LT 0.01	3.26 (9,956)	LT 0.01
Distal Latency	1.48 (1,325)	0.22	1.35 (3,323)	0.26
Amplitude Ratio	4.62 (1,332)	LT 0.03	4.94 (3,330)	LT 0.01
MCV	9.20 (1,329)	LT 0.01	4.50 (3,327)	LT 0.01
Sural Sensory				
*Latency, Amp., SCV	3.83 (3,321)	LT 0.01	2.14 (9,953)	LT 0.02
Latency	1.18 (1,325)	0.28	1.17 (3,323)	0.32
Amplitude	0.22 (1,323)	0.64	2.11 (3,321)	LT 0.01
SCV	6.69 (1,325)	LT 0.01	3.10 (3,323)	LT 0.03

*Variables used in MANCOVA

Table 8-5
Summary of Peroneal MCV for Cumulative Exposure Index by Length of Employment

Length of Employment		Cumulative Exposure Index (ppm months)		
		500-1000	1000 - 1500	GT 1500
LT 10 years	$\overline{X}$ (sd)	44.5 (4.0)	43.9 (4.4)	40.3 (-)
	n	46	3	1
10 - 20 years	$\overline{X}$ (sd)	44.6 (3.9)	45.2 (5.9)	40.2 (5.8)
	n	26	16	8
GT 20 years	$\overline{X}$ (sd)	45.4 (0.2)	41.4 (0.5)	39.4 (4.9)
	n	2	2	20

ANOVA Results:

Source of Variation	F-Value (df)	Pr GT F
Rows (Length of Employment)	12.9 (2,115)	0.73
Columns (Cum. Exp. Index)	122.8 (2,115)	LT 0.05
Interaction	23.8 (4,115)	0.88

Table 8-6: Summary of "No" Action Potential Results by Exposure Group

	YES		NO		X^2	P
	N	%	N	%		
Peroneal Motor						
Comparison	202	94.0	13	6.1		
DL Group	44	100.0	0	0.0	3.85	0.28
M Group	59	93.7	4	6.4		
DH Group	38	90.5	4	9.5		
All Exposed	141	94.6	8	5.4	0.07	0.79
Sural Sensory						
Comparison	200	93.5	14	6.5		
DL Group	39	88.6	5	11.4	15.12	LT 0.01
M Group	62	98.4	1	1.6		
DH Group	33	78.6	9	21.4		
All Exposed	134	89.9	15	10.1	1.49	0.22

Table 8-7: Summary of PNS Positive Responses to Neurological History Questionnaire: Exposed vs. Controls

Symptom	Control		DL		M		DH		Exposed	
	n	%	n	%	n	%	n	%	n	%
Muscle Weakness (Paresis)	30	14	8	18	12	20	6	15	26	18
X^2					1.32				0.99	
P					0.73				0.32	
Trembling or Shaking Hands	38	18	14	32	15	25	8	20	37	26
X^2					4.81				2.99	
P					0.19				0.08	
Difficulty in Walking	17	8	5	11	6	10	2	5	13	9
X^2					1.30				0.09	
P					0.73				0.76	
Unexplained Numbness or Tingling in Hands or Feet	32	15	8	18	8	13	12	30	28	19
X^2					6.08				1.10	
P					0.11				0.30	
Pain in Either Leg	51	24	9	20	14	23	9	23	32	22
X^2					0.29				0.19	
P					0.96				0.66	

CHAPTER 9 - BEHAVIORAL EXAMINATION

A. INTRODUCTION:

Earlier studies of workers chronically exposed to CS_2 suggested impairment of psychomotor speed and coordination, memory, and visual perception[3,8]. Slight personality changes were also seen in the exposed workers. More severe impairments were identified in workers with verified or suspected CS_2 poisoning[4]. In a later study by the same author, retardation of psychomotor speed, impairment of motor coordination and depressive mood were found to be the most valid indicators of CS_2 exposure[5]. Tuttle et al.[15] found that calculated estimates of CS_2 exposure were correlated with impaired performance on tests of visual reaction time, manual dexterity, rate of visual search and perceptual organization. Visual impairments were also noted by Szymankova[14]. The CS_2 exposure levels in all of these reports ranged from moderately low (5 ppm) to concentrations in excess of 20 ppm.

On the basis of such findings, NIOSH chose a battery of tests that would assess the psychological, psychomotor, sensori-perceptual and memory disorders thought to be associated with chronic and acute CS_2 exposures, and which could be readily administered to a large group of workers. The behavioral data presented in this chapter include results of (1) psychological and neurological questionnaires, (2) psychomotor examinations, and (3) visual function tests.

B. METHODS AND MATERIALS:

1. Subjects

From the original 273 potentially exposed workers, 209 Caucasian workers signed the informed-consent statement and met the initial study criteria. However 32 of these workers withdrew before the study started, leaving a sample size for testing of 177 exposed workers or 65% of those originally available. Of the 422 potential controls, only 233 Caucasians (55% of the workers) fit the initial study criteria, signed the consent form and were available to participate in the study. The general methods of case and control selection, testing, and data analysis have been presented earlier (see Chapters 2, 3, 4, 5).

After the data were collected, additional editing was necessary to eliminate the data of those workers which were judged to have health characteristics and personal habits that could potentially confound the subsequent data analyses. Workers found to possess or indicate the following conditions were eliminated from all statistical analyses:

-had blood lead levels greater than 40 ug/dl
-indicated on questionnaire a diagnosis of diabetes mellitus
-indicated use of medicine for high blood/urine sugar
-indicated use of stimulants, sedatives or tranquilizers
-indicated use of marijuana, cocaine or similar types of drug
-indicated consumption of more than 35 alcoholic beverages per week

Since there were only 12 non-white individuals in the exposed group, the final analysis was limited to whites. The final sample size used in the analyses consisted of 131 exposed, or 48% of those originally available and 167 controls, 40% of those originally available.

2. Test Procedures

All psychological testing was performed by eight experienced NIOSH staff members and a consultant from the University of Michigan during a consecutive three-week period. All participants were given a physical examination and administered a health history questionnaire, which included items pertaining to behavioral and neurological symptoms. Workers were tested before their work shift began, approximately 16 hours after their last potential CS_2 exposure. The scheduling of persons to be tested was arranged so that personnel administering the tests were unaware of each subject's exposure history.

3. Psychological Tests

The Profile of Mood States (POMS) described by McNair et al.[9] and an abbreviated mania scale from the Minnesota Multiphasic Personality Inventory (MMPI)[11] were included to assess the possible psychological effects of CS_2 exposure. The POMS consisted of 65 adjectives describing mood states; the MMPI consisted of 13 questions involving activities. Taken together, these two scales measured each worker's response to six identifiable mood or affective states: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, confusion- bewilderment and mania or excitability.

4. Psychomotor Tests

Performance measures of eye-hand coordination and reaction time to visual stimulus were obtained for each worker using tests previously described by NIOSH[7]. The eye-hand coordination test (Michigan version) required the worker to follow a weaving, maze-like path with an electronic recording stylus. At each of the 119 points in the path, the stylus had to be fully inserted to the bottom of a hole, which required the execution of a series of discrete arm movements. The test is sensitive to both the presence of tremor and impaired vision, both possible consequences of CS_2 exposure. The mean time to move the stylus from hole to hole, and the variability in interhole movement time constituted the performance scores for this test.

Reaction time to a visual stimulus was measured using an eight-choice reaction time apparatus. The required response to each stimulus was to move the index finger of the preferred hand a fixed distance in order to extinguish a stimulus lamp. The apparatus was designed so that two performance measures, simple reaction time (RT) and choice RT, could be obtained in response to each stimulus. The time interval between the onset of one of the eight stimulus lamps and the release of the "home" key served as a measure of simple reaction time; the interval between the onset of a stimulus lamp and the subsequent response to the lamp was the measure of choice reaction time. Number of errors was also recorded.

5. Sensori-Perceptual Tests

The Neisser test[10] was used to measure the rate of visual search. The test requires the subject to search for target letters on a sheet of paper containing rows of random arrangements of letters. Each worker was given one 20-second training trial followed by four test trials. In two of the test trials, which lasted 30 seconds each, the target was either one of two letters; the remaining two trials were 20 seconds each and involved a search for a single letter. The performance score was the average number of targets detected per second. This test has been found to be sensitive to the effects of CS_2[15], as well as other neurotoxic agents[18].

Visual acuity, depth perception and color vision were measured using a standard Bausch and Lomb Orthorator^{R}. Each worker's corrected acuity for both far and near vision was assessed using the standard 20/20 reference. The Orthorator assessed depth perception by determining the minimal angle of separation between two objects at an apparent distance of six meters from the retina. Measurement units for depth are scaled in terms of the minimal angle of stereopsis. The value is commonly expressed as a visual angle in seconds: the smaller the angle, the better the depth perception. Color vision was judged as being normal or abnormal based on the correct identification of five of the six color slides presented by the orthorator.

In the absence of pre-exposure acuity data for each worker, information on current visual functions was used primarily as a control procedure to identify and screen out workers with uncorrected visual impairments. Such unidentified visual impairments can serve to confound the results from the performance tests which are vision-dependent.

6. Memory Tests

The short-term memory capacity of the worker was assessed with the memory span digit test[2]. Lists of digits, selected randomly from the set of numbers 0 through 9, were presented sequentially on a video display. Workers were instructed to memorize the list of digits and to then recite this list of digits in the same order when a question mark appeared on the video display. The testing sequence consisted of two blocks of trials. The first block was used for practice and to establish the lengths of the list to be tested in the subsequent test block. On each of the 40 trials comprising the test block, the examiner was required to compare the worker's digit recitation against the correct sequence and determine the accuracy as well as the number, type, and location of the errors. A probit analysis was then used to provide an estimate of the length of the list that each worker could corectly recite 50% of the time. This value served as an estimate of the worker's short-term memory capacity.

7. Health Questionnaire/Neurological History

After the above tests were completed, each worker was escorted to a separate test area where trained interviewers obtained an occupational background and health history from each participant. A component of the questionnaire dealt with certain health or neurologic symptoms reported by the worker. Eleven of the items had direct behavioral or performance implications: 1) muscle weakness, 2) blurred vision, 3) trembling hands, 4) memory difficulty, 5) walking difficulty, 6) dizziness, 7) sleeping difficulty, 8) frequent fatique, 9) headaches, 10) numbness of hands or feet, and, 11) mental conditions. The interviewer asked each worker if he ever had or was currently bothered with each of the symptoms, and if so, when. The data consisted of each worker's positive or negative report of having recently or presently experienced each symptom.

8. Data Analysis

The experimental design consisted of three exposed sub-groups with a total of 131 workers, and a comparison group of 167 workers. The three exposed sub-groups were:

(a) the definitely-low exposed group (DL), 41 workers,
(c) the moderately exposed group (M), 54 workers, and
(b) the definitely-high exposed group (DH), 36 workers.

A Chi Square test was used to analyze data from the health symptom questionnaire; analysis of covariance was used to anmalyze the data from the performance tests. A 5% significance criterion level was chosen. With the exception of the memory test, where education level was also used, age, which was also directly related to length of employment, was used as the covariate for the behavioral test data. For the analysis of the health questionnaire data, age was introducted in the Chi square analysis as an additional variance source. Three groups were formed consisting of those workers a) under 30 years, b) between 30 and 40 years and c) 41 years and older. In this manner, the effect of age was separated from the measure of effect, and thereby reduced the potential confounding between the reported health symptoms and the worker's age.

C. RESULTS:

1. Group Characteristics

Table 9-1 shows the distribution of ages among the exposure groups for the workers used in analysis of symptoms. Group characteristics for other variables are summarized in Table 9-2. On the average, workers in the comparison group were significantly younger than the workers in the three exposure groups, ($F_{[3, 294]} = 4.42$, p less than 0.05). There was, however no statistical difference in the mean ages of the three exposure groups. There was also a statistically significant difference between the comparison and exposure groups in the average number of years of employed with the company (8.6 years for comparison subjects vs. 11.8 for DL, 12.1 for M, and 14.0 for DH, $F_{[3,294]} = 12.1$, p less than 0.05). Again, no statistical difference was found among the employment lengths of those workers belonging to the three exposure groups. Finally, no statistically significant difference was found in the number of years each group attended school, ($F_{[3,293]} = 2.31$, p 0.05). As expected, age and employment length were significantly correlated, ($r = 0.67$, p less than 0.05, $n = 371$). The data indicated that the workers comprising the three exposure groups were relatively homogeneous with respect to age and employment length, but different from the comparison group.

The groups were also analyzed for differences in potential exposure based on the computed "exposure index". As expected, all groups with the exception of the moderate and definitely low exposure groups were significantly different from each other ($F_{[3,294]} = 85.7$, p LT 0.05). The results from exposure index computation, which was based on the work history of previous years, also served to substantiate the initial groupings of the exposed workers, which was based on current work exposures, i.e., at the time of the survey.

Packs of cigarettes smoked per year for the comparison group (349) was not statistically significantly different from that of the three exposed groups, which averaged 323, 324, and 282 packs per year for the

DL, M, and DH groups, respectively ($F_{[3,286]} = 0.87$, p GT 0.05). In addition, the self reported alcoholic consumption of the exposed groups was not statistically different from the comparison group, ($F_{[3,293]} =$ 0.43; p GT 0.05).

2. Summary of Health/Neurological Questionnaire

Table 9-3 lists, by exposure category, the percent of positive responses for each of the 11 items composing the relevant symptoms of neurological impairment. The exposed sample was subdivided into three smaller groups, reflecting the average exposure level for the worker. Reporting of blurred vision by the exposed workers showed a dose/response relationship and a statistically significant difference between the comparison and exposed groups. Five other symptoms were found to be statistically different between the exposed and comparison groups, namely: trembling of hands, memory difficulty, dizziness, insomnia and fatigue. Blurred vision and dizziness were primarily evident in the high exposure group, with nearly 33% of those workers reporting blurred vision and 42% reporting dizziness.

With the exception of hand trembling, age did not affect the prevalence pattern established by the exposure conditions. Moreover, when the symptom of hand trembling was analyzed for age effects, significantly more of the younger exposed workers (41%) reported frequent hand trembling than did their age mates (20%) in the comparison group. Furthermore, of the 29 exposed workers 30 years of age or younger, eight were in the high exposed group and 62% of them reported the symptom of hand trembling, whereas 40% of the young men in the moderately exposed group reported the same symptoms and 27% of the young men in the low exposed group reported frequent hand trembling. In all cases of the reported effects, the Chi square statistic was significant at the 5% level or better.

3. Psychological Tests

To compensate for the significant age differential in favor of the younger comparison group, all values in Table 9-4 were adjusted for age, lowering the score values for the comparison group. The analysis of the excitability dimension from the POMS and the mania scale from the MMPI revealed no significant difference on any of the scales (p greater than 0.05).

4. Neisser Test

The comparison group correctly detected an adjusted mean of 0.52 targets per second (SD = 0.11 targ/sec) or a total of 52 targets during the 100-sec search period (Table 9-4). The exposure groups detected an average of 0.48, 0.52, and 0.48 targets per sec (SD = 0.12 targ/sec) on the same test. A statistically significant difference was found in the overall analysis of the adjusted means for the four groups ($F_{[3,288]}$= 2.71, p less than 0.05). In the subsequent mean tests, the mean for the comparison group was statistically different from the means characterizing the the DH and DL groups. The mean of the M group was also significantly different from that of the DL group; the M group's mean was not statistically different from the comparison group's.

Figure 9-1 shows the relatively high detection rate for the medium exposure group, as compared with the other two exposure groups. Figure 9-1 also shows the effects of task difficulty and practice on the number of targets detected per sec for the comparison group and the three exposure groups. As expected, search time per target was affected by the number of targets searched, as shown in Figure 9-1, but task performance was not affected to a significant degree by experience or training on the task. For example, when the target stimulus was one letter, there was little or no improvement in the number of targets detected per second between the first and second trials. Moreover, when the search was for two letters, performance declined slightly for the second trial.

5. Memory Span

Examination of Table 9-4 shows that the comparison group had an average memory span of 5.40 digits, whereas the workers who comprised the three exposed groups registered mean adjusted memory spans of 5.04, 5.35, and 5.31 digits for the DL, M, and DH groups, respectively ($F_{[3, 289]}$ = 2.10, p greater than 0.05). Both education and age were used as covariates. The correlation between age and memory span was -0.24 and a 0.28 between education and memory span. The overall correlation between exposure and memory span was only -0.15.

6. Simple/Choice Reaction Time

The adjusted mean simple RT, shown in Table 9-4 for the comparison group was 0.23 sec and the mean simple RT for the three exposure groups was 0.18, 0.21, and 0.22 sec for the DL, M, and DH groups. The SDs for all groups were similar, approximately 0.10 sec. Mean data for the choice RT for comparison and exposed groups were also similar: 0.33 sec for controls and 0.38, 0.33, and 0.35 sec for the DL, M, and DH groups ($F_{[3,291]}=2.19$, p greater than)$_{0.05}$).

7. Eye-hand coordination

The mean interhole latency, adjusted for age, was 0.51 sec for the comparison group (Table 9-4). By comparison, the exposed groups had mean interhole latencies of 0.54, 0.51, and 0.52 sec for the DL, M, and DH groups, which were not significantly different ($F_{[3,292]} = 2.07$, p greater than 0.05). An analysis of the variation in movement time was also performed. Significantly more variation was found in the low exposure group (0.15 units of variance) than in the other groups ($F_{[3,292]} = 4.50$, p less than 0.05) but the values did not show a dose-response relationship.

8. Visual Acuity Tests

The analysis of the binocular visual acuity data indicated that there were no significant differences in the corrected vision between the controls and the exposed for both the far and near tests. For all groups, the average corrected acuity was equivalent to the 20/20 standard. Color vision was normal for all groups.

The values in Table 9-4 for depth perception are scaled according to the minimal angle of stereopsis that the subject could detect. For example, the comparison group registered a mean of 81.7 sec of visual angle. The DL group exhibited a somewhat higher level of depth perception, 57 sec/angle; the DH group showed the poorest depth perception, 113.6 sec/angle. However, these differences were not statistically significant, ($F_{[3,288]} = 2.26$, p greater than 0.05).

D. DISCUSSION AND CONCLUSION:

The findings from the performance tests and the questionnaire phase of this study appear to be at odds. For example, rayon workers reported significantly more symptoms (six of eleven items composing the health questionnaire) than did the non-rayon workers. Yet these same workers differed significantly from non-rayon workers on only two of seven performance measures. The exposed workers represent a sample of men who had worked at least one year, and as many as 31 years prior to the study, in a plant with average exposures below 20 ppm.

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Table 9-1 Number of Workers for Analysis of Symptoms by Age Category and Exposure Group

Age Range	Comparison	Exposed			
		Low	Medium	High	Total
Less than 30	61 (36 %)	11 (8 %)	10 (8 %)	8 (6 %)	29 (22 %)
30 - 40	65 (39 %)	19 (14 %)	27 (21 %)	11 (8 %)	57 (44 %)
Greater than 40	41 (25 %)	11 (8 %)	17 (13 %)	17 (13 %)	45 (34 %)
All ages	167 (100 %)	41 (8 %)	54 (13 %)	36 (13 %)	131 (100 %)

Table 9-2: Study Group Characteristics

	Exposed				Comparison
	DL	M	DH	All	
N	41	54	36	131	167
Age - mean	37.3	37.7	39.4	38.1	34.3
- S.D.	10.7	9.2	10.7	10.1	8.6
Employment[1]	11.8	12.1	14.0	12.5	8.6
- S.D.	7.4	5.7	7.4	6.8	4.9
Smoking[2]	323	324	282	312	349
- S.D.	223	234	220	226	233
Drinking[3]	1.8	2.0	1.9	1.9	2.6
- S.D.	4.7	4.9	6.6	5.3	5.7
Education	10.5	10.3	10.4	10.4	11.0
- S.D.	2.1	2.0	1.6	1.9	1.7

Notes:
1. Employment time with company.
2. Mean packs smoked per year.
3. mean number of drinks per week.

Table 9-3: Prevalence (Percent) by Exposure Group of Symptoms Reported on Health Questionnaire

Ill Health	Exposed			Comparison	Effect	
Symptoms	DL	M	DH		Exposure	Age
N less than or =	41	54	36	167		
Muscle Weakness	17.07	16.67	17.14	10.18	4.08	0.50
Blurring Vision	7.32	11.11	33.33	6.59	15.48*	1.38
Trembling Hands	29.27	20.37	22.22	13.77	10.00*	6.62[a]
Memory Difficulty	31.71	22.22	47.22	13.86	19.52*	4.27
Walk Difficulty	9.76	5.56	5.56	6.63	1.11	3.19
Dizziness	17.07	16.67	41.67	10.78	18.34*	2.54
Insomnia	39.02	48.15	44.44	20.36	18.54*	0.27
Fatigue	53.66	37.04	50.00	17.96	27.45*	0.34
Headaches	19.51	9.26	19.44	8.43	7.01	2.91
Numbness	14.63	14.81	30.56	12.57	6.07	0.19
Mental Condition	0.00	7.55	0.00	1.20	4.09	0.77

* Statistical significance at p less than 0.05.

[a] Significant age effect at p less than 0.05.

Table 9-4: Psychological Test Results for Exposed and Comparison Groups

Performance Tests	Units		Exposed DL	M	DH	Comparison	F Test
N less than or =			41	54	36	167	
POMS[1]		$\bar{X}$	15.78	16.52	15.74	17.19	1.06
		SD	5.74	5.70	4.56	5.52	
		$\bar{X}^c$	15.80	16.55	15.79	17.13	
MMPI[2]		$\bar{X}$	8.13	7.87	7.37	7.60	0.32
		SD	4.36	4.05	5.17	4.14	
		$\bar{X}^c$	8.18	7.49	7.48	7.53	
Neisser	Targ/Sec	$\bar{X}$	0.48	0.52	0.48	0.52	2.71*
		SD	0.12	0.10	0.14	0.11	
		$\bar{X}^c$	0.48	0.52	0.48	0.52	
Memory[3]	Digits	$\bar{X}$	5.04	5.35	5.31	5.40	2.10
		SD	0.79	0.74	0.73	0.94	
		$\bar{X}^c$	5.04	5.35	5.31	5.40	
Choice RT	Secs	$\bar{X}$	0.38	0.33	0.35	0.33	2.19
		SD	0.12	0.11	0.12	0.12	
		$\bar{X}^c$	0.38	0.33	0.35	0.33	
Simple RT	Secs	$\bar{X}$	0.18	0.21	0.22	0.23	2.70
		SD	0.09	0.09	0.11	0.10	
		$\bar{X}^c$	0.18	0.21	0.22	0.23	
Eye Hand	Secs	$\bar{X}$	0.54	0.51	0.52	0.51	2.07
		SD	0.08	0.06	0.07	0.07	
		$\bar{X}^c$	0.54	0.51	0.52	0.51	
Eye Hand Variance		$\bar{X}$	0.15	0.13	0.13	0.13	4.60*
		SD	0.07	0.03	0.03	0.03	
		$\bar{X}^c$	0.15	0.13	0.13	0.13	
Depth	Sec/Angle	$\bar{X}$	59.2	111.74	117.35	79.05	2.26
		SD	87.40	137.74	138.72	109.67	
		$\bar{X}^c$	57.12	108.84	113.59	81.75	

a F statistics compared Comparison and exposed subsets.
b Positive relationship between score value and prevalence of symptom.
c All scores except memory adjusted for age; memory scores adjusted for age and education.
1 POMS Scale Vigor/Activity 2 MMPI Mania Scale
3 Only complete data sets analyzed, with education covariate.
* p less than 0.05, one-tailed test.

CHAPTER 10 - EFFECTS OF CARBON DISULFIDE ON THE ENDOCRINE SYSTEM

A. EFFECTS OF CS_2 ON THE THYROID GLAND

1. Literature Review

In 1971, Cavalieri et al.[1] reported a study of several endocrine systems in a group of hospitalized people chronically intoxicated with CS_2. Part of this study was a thyroxin assay in 68 workers and 15 controls. The mean serum thyroxin was 3.43 ± 0.07 mcg percent in the exposed and 4.60 ± 0.19 in the control group. This difference was "highly significant." The achilles-tendon reflex time was also reported to be lengthened significantly in the exposed group.

Cavalieri[2] also investigated an Italian cohort of 45 exposed and 18 unexposed subjects for serum thyroxin, cholesterol, triglycerides, total lipids, and lipoproteins. Average serum thyroxin of exposed subjects was 3.0 mcg/100 cc and 4.5 mcg/100 cc for control subjects. The difference was statistically significant at p less than 0.01. Also reported was a signficant reduction of serum thyroxin with increased duration of exposure. Although serum cholesterol and triglycerides showed no significant changes, there was an inverse relation between serum thyroxin (diminished) and serum cholesterol (increased).

2. Methods

Thyroid function was determined by triiodothyronine uptake (T_3), serum thyroxin by radioimmunoassay (RIA, T_4), and T_3 - T_4 index (T_7); if T_4 was found to be abnormal, a thyroid stimulating hormone analysis (TSH) was determined. Serum for this work was obtained at the same time as that drawn for lipid studies (see Chapter 6).

3. Results

a. By Current Exposure

Table 10-1 shows the statistical analysis of the thyroid function data. There is a significant difference in T_3 uptake between the control and exposed groups and between the control and DL, M, DH groups. Although there is a decrease in T_3 uptake as exposure increases, the differences are not statistically significant. When considered as a set through multivariate analysis of covariance (MANCOVA), T_3, T_4, and T_7 thyroid function tests are significantly associated with exposed vs. unexposed status, but not with exposure subgroup status.

b. By Cumulative Exposure

Figures 10-1 through 10-3 are scattergraphs of T_3, T_4, and T_7 vs. log(exp. ind.). Table 10-2 shows T_3, T_4, and T_7 thyroid function tests regressed on log(exp. ind.), age, and the interaction of age and log(exp. ind.); the cell at the bottom shows F and P values for MANCOVA analysis of the set of three thyroid function tests, taken together. Age explains a small, but statistically significant amount of the variance of T_3, but there are no significant correlations of thyroid function tests with log(exp. ind.) or with the interaction of age with log(exp. ind.); nor is there a significant association with cumulative exposure index when the set of thyroid function tests is considered as a group, using MANCOVA.

4. Discussion

Previous studies have directly shown that chronically CS_2 poisoned workers have diminished serum thyroxin levels, sometimes to the level of clinical hypothyroidism.[1,2] This study was planned to determine whether workers exposed to CS_2 at concentrations of 20 ppm or less show diminished serum thyroxin levels. If such diminution were found, then serum thyroid stimulating hormone (TSH) was to be measured to determine whether CS_2 caused a primary or secondary (hypothalamic) effect on the thyroid gland.

The tables show that serum thyroxin, as measured by RIA, was not lowered in those workers exposed to CS_2. The T_7 level, which represents free biologically active thyroxin, also showed no significant differences between groups. Since these values were not abnormal in the exposed group, TSH determinations were not needed. The tables also show that the T_3 uptake is significantly lower (p less than 0.01) in the exposed group than in the control group. Analysis by cumulative exposure shows that this difference is due to the slight age difference between the groups, not to CS_2 exposure.

At the exposure levels of CS_2 common in this cohort, there is no observable effect on the thyroid gland. The most likely reason for this difference from earlier studies is dose-related; the earlier studies used chronically intoxicated subjects.

B. EFFECTS OF CS_2 ON CARBOHYDRATE METABOLISM

1. Literature Review

Finulli and Ghislandi[4] reported an apparent excess of diabetes among patients hospitalized in Milan "with vascular diseases due to chronic exposure to CS_2 vapors."

In 1969, Ferrero[3] reported an increased incidence of diabetes (4 cases) in a cohort of 67 workers exposed to CS_2. It appears that this was a cluster of occupations noted in the course of clinical practice, not a finding in a prospective cross-sectional study.

In Goto's[9] 1971 study of 195 Japanese CS_2-exposed workers and 39 controls, prednisolone-glucose tolerance testing (P-GTT) was used in an attempt to evaluate a diabetogenic effect of CS_2. Fasting blood glucose values for exposed and control groups were similar, but the one- and two-hour glucose samples were both significantly higher in the exposed group (both p less than 0.01). When length of exposure was taken into account, glucose levels were higher in those exposed for longer periods of time.

Goto et al.[6] studied 68 Yugoslavian viscose rayon workers and seven controls in 1972 with a modified P-GTT, taking only fasting and two-hour blood sugars. No significant differences were found.

Sugimoto et al.,[10] in a five-year follow-up of Goto's 1971 cohort, found significantly higher glucose levels at the one-hour point in a P-GTT.

Franco et al.[5] studied 66 CS_2-exposed workers and 66 controls with an oral GTT augmented by 5-methyl prednisolone. They reported significantly higher glucose levels among the exposed group at 30, 60, 90, and 120 minutes after a glucose load. Fasting and 180 minute levels were higher, but not significantly different.

The Finnish group, by contrast, found no difference in plasma glucose in the 1967 and 1972 Finnish studies.[7,8]

2. Methods

Only the fasting blood sugar was determined. Means and standard deviations of the exposed and unexposed groups were compared and the fasting blood sugar was regressed on age, log(exposure index), and the interaction of age and log(exp. ind.). Workers with a history of taking medicine for high blood sugar or sugar in the urine, or who were taking ACTH or corticosteroids were excluded from analysis.

3. Results

Results of analysis by current exposure are given in Table 10-3; there are no significant differences in fasting blood sugar between groups. Results of analysis by cumulative exposure are given in Table 10-4; there is no significant correlation between fasting blood sugar and any of the independent variables considered.

4. Discussion

The lack of correlation between exposure and blood glucose in this cohort adds very little to our knowledge of the toxicology of CS_2. In designing the study, we decided that other components were of more importance than a precise study of effects on carbohydrate metabolism and that we could not do glucose tolerance testing without sacrificing some other portion of the study. The design included a fasting blood sugar, since the test could be done on blood drawn primarily for other purposes and might detect an extreme effect, if present.

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D. SUMMARY

To evaluate the effect of CS_2 on thyroid function, determinations of triiodothyronine uptake (T_3), serum thyroxin by RIA (T_4), and T_3-T_4 index (T_7) were made. A significant difference in T_3 uptake was observed between the control and exposed groups and between the control and DL, M, DH groups. The decrease in T_3 uptake as exposure increases was not found to be statistically significant. Age explained a small, but statistically significant amount of the variance of T_3 but there were no significant correlations of thyroid function tests with log(exp. ind.) and the interaction of age with log(exp. ind.).

To evaluate the effect of CS_2 on carbohydrate metabolism, mean fasting blood sugar levels and standard deviations of the exposed and unexposed groups were compared across groups, and the fasting blood sugar was regressed on age, log(exposure index), and the interaction of age and log(exp. ind.). Workers with a history of taking medicine for high blood sugar or sugar in the urine, or who were taking ACTH or corticosteroids were excluded from analysis. No significant differences between groups, nor any significant correlation between fasting blood sugar and log(exp. ind.) were found.

TABLE 10-1: Thyroid Function By Current Exposure Category
(Control versus DL, M, DH, and All Exposed)

	Control	DL	M	DH	All Exposed
N	216	43	58	43	144
T4 by RIA $\bar{x}$	9.44	9.66	9.43	9.31	9.46
S.D.	1.88	1.49	1.59	1.82	1.63
P value			0.83		0.92
T3 Uptake $\bar{x}$	38.29	37.91	37.24	37.19	37.42
S.D.	3.13	2.12	2.90	2.97	2.71
P value			0.03*		LT 0.01*
T7 Index $\bar{x}$	3.59	3.66	3.49	3.45	3.53
S.D.	0.73	0.57	0.54	0.83	0.58
P value			0.38		0.35
T4, T3, T7 - F			1.32		2.73
P value			0.22		0.04*

* Significant at 0.05 level.

Table 10-2: T_3, T_4, and T_7 Thyroid Function Tests Regressed on Log(Exposure Index), Age, and Age · Log(Exposure Index)

Predictor	Estim. of Regression Coefficient	t	Pr[GT t]	Model R^2 $F_{3,378}$*	Partial R^2 $F_{2,378}$*
T_3 uptake					
(Intercept)	41.061	33.80	0.0001	0.417	0.006
Age	-0.0766	-2.20	0.03	5.48	1.17
Log(Exp Ind)	-0.7194	-1.25	0.21		
Age · Log(Exp Ind)	0.0134	0.91	0.36		
T_4 by RIA					
(Intercept)	8.4485	11.29	0.0001	0.006	0.003
Age	0.0306	1.43	0.15	0.77	0.55
Log(Exp Ind)	0.3049	0.86	0.39		
Age · Log(Exp Ind)	-0.0091	-1.00	0.32		
T_7 Index					
(Intercept)	3.4073	12.03	0.0001	0.004	0.004
Age	0.0063	0.78	0.44	0.50	0.67
Log(Exp Ind)	0.0867	0.64	0.52		
Age · Log(Exp Ind)	-0.0031	-0.91	0.36		

*Pr[$F_{3,120}$ GE 2.60] = 0.05; Pr[$F_{2,120}$ GE 3.00] = 0.05

T_3, T_4, T_7	F	P
Age	1.61	0.18
Log(Exp Ind)	0.52	0.67
Age · Log(Exp Ind)	0.51	0.68

Table 10-3: Fasting Blood Sugar (FBS) By Current Exposure Category (Control versus DL, M, DH, and All Exposed)

	Control	DL	M	DH	All Exposed
N	212	43	59	43	145
$\bar{x}$	84.27	87.47	85.81	85.07	86.08
S.D.	11.85	12.89	12.02	9.43	11.56
P value			0.40		0.15

Table 10-4: Fasting Blood Sugar Regressed on Log(Exposure Index), Age, and Age · Log(Exposure Index)

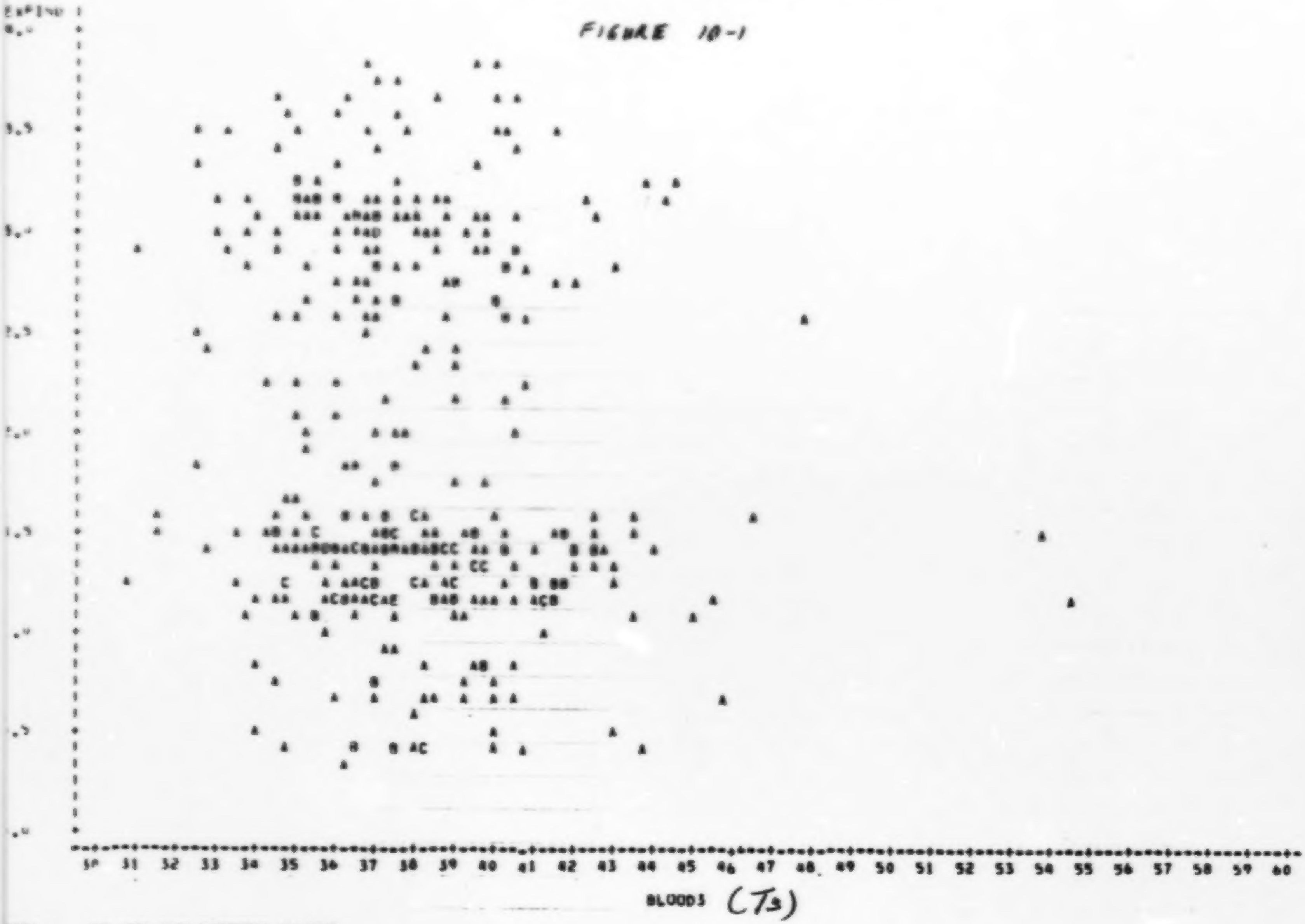
Predictor	Estim. of Regression Coefficient	t	Pr[GT t]	Model R^2 $F_{3,378}$*	Partial R^2 $F_{2,378}$*
(Intercept)	83.673	16.51	0.0001	0.015	0.004
Age	0.0041	0.03	0.98	1.92	0.70
Log(Exp Ind)	-1.1222	-0.47	0.64		
Age · Log(Exp Ind)	0.0484	0.78	0.43		

0-1

STATISTICAL ANALYSIS SYSTEM   [illegible] FRIDAY, JULY 17, 1981   1

PLOT OF LEXPIND*BLOODS   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 10-1



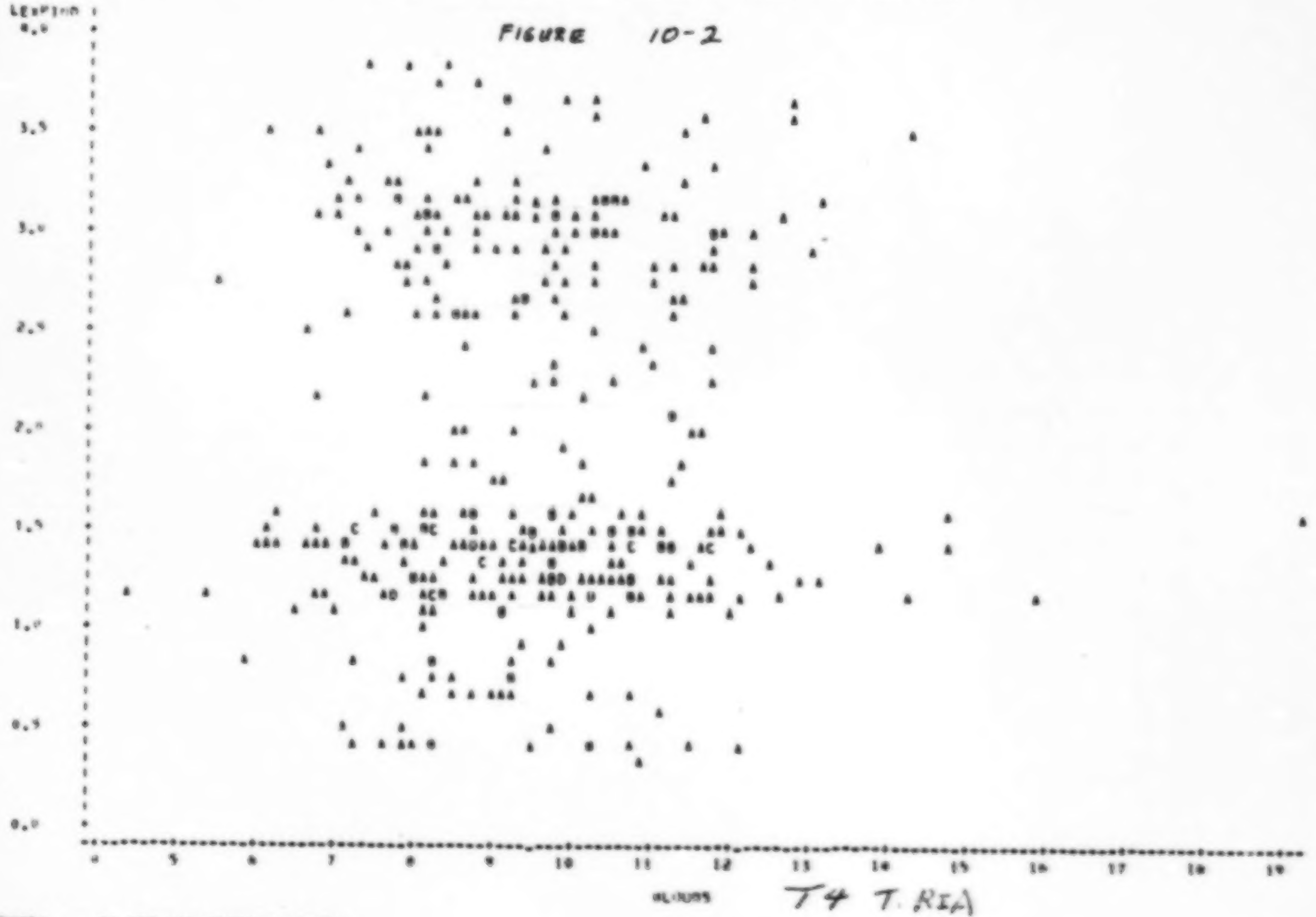
TE: 27 OBS HAD MISSING VALUES

10-2

STATISTICAL ANALYSIS SYSTEM   10:00 FRIDAY, JULY 17, 1981   3

PLOT OF LEXPIND*BLOODS   LEGEND: A = 1 OBS, B = 2 OBS, ETC.

FIGURE 10-2



T4 T.RIA

NOTE: 27 OBS HAD MISSING VALUES

10-3

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STATISTICAL ANALYSIS SYSTEM                    14:00 FRIDAY, JULY 17, 1981   2
PLOT OF LEXPIND*BLOODA    LEGEND: A = 1 OBS, B = 2 OBS, ETC.
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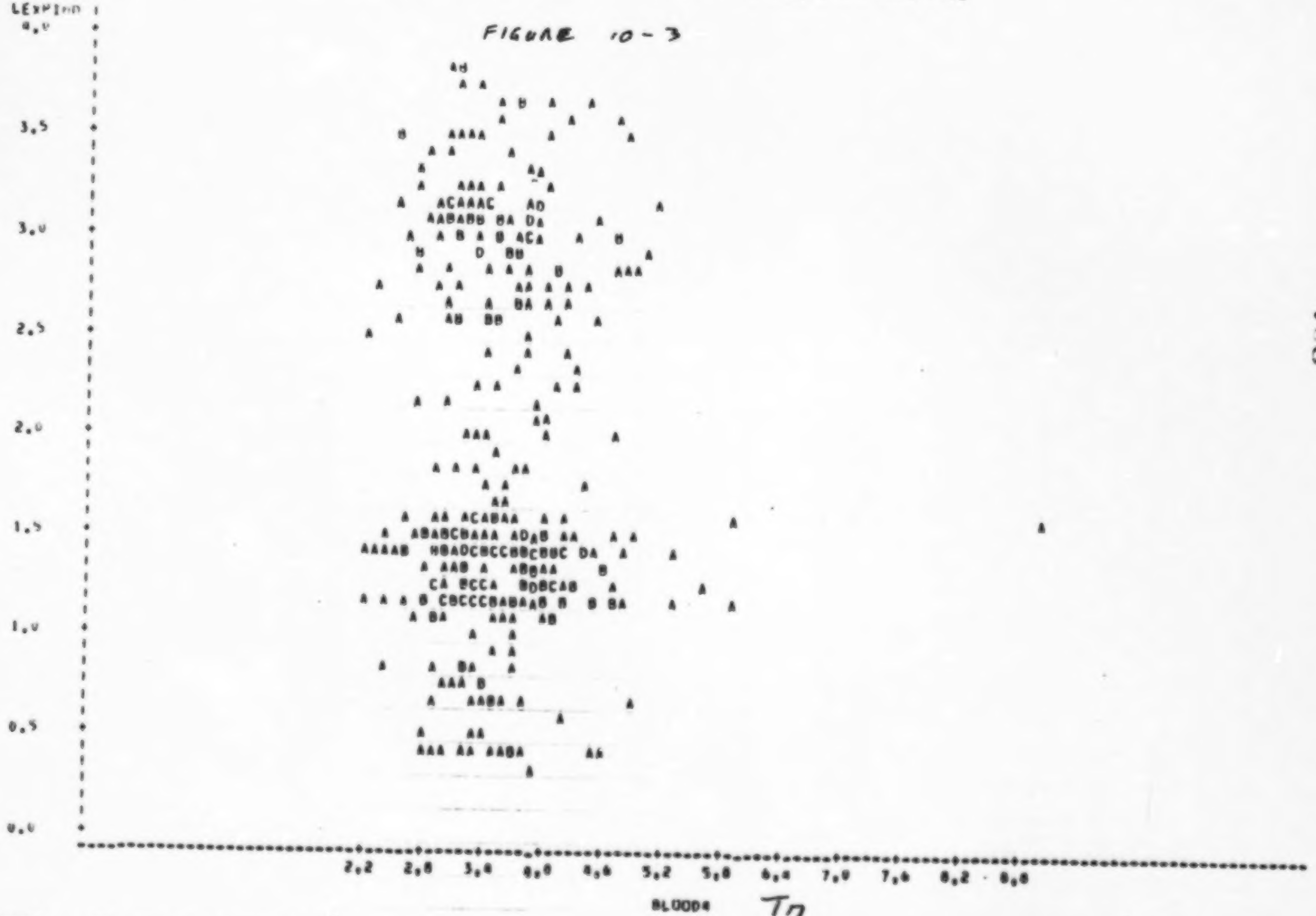
FIGURE 10-3



T7

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NOTE:    27 OBS HAD MISSING VALUES
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201<

CHAPTER 11 - EFFECTS OF CARBON DISULFIDE ON THE REPRODUCTIVE SYSTEM

A. LITERATURE REVIEW

In 1971, Maugeri et al. studied chronically CS_2-intoxicated Yugoslavian workers[4]. By determining total and fractionated urinary 17-Ketosteroid excretion, they detected diminished testicular hormone production[1] which was not reversed by administration of chorionic gonadotropins. In the same study, they discovered decreased urinary testosterone and gonadotropin excretion. This information led them to postulate both a primary and secondary effect of CS_2 on the testis.

Lancranjan, having discovered adrenal and gonadic insufficiency in workers with chronic carbon disulfide poisoning,[2] investigated germinal insufficiency in particular.[3] Her cohort consisted of 140 CS_2 exposed workers with an duration of average exposure of 40 ± 25 months and 50 control workers. One hundred thirty-three of the 140 exposed workers had the diagnosis "chronic carbon disulfide poisoning". The exposed group had a more frequent history of "sexual dynamic disorders" and decrease of libido; their semen analyses showed significant increases in asthenospermia (decreased motility), hypospermia (decreased count) and teratospermia (abnormal morphology) as compared with controls (all significant at p less than 0.001). Evaluation of 17 Ketosteroid secretion revealed leydigian (androgen) insufficiency in those subjects with spermatogenic disorders.

Total urinary gonadotropins in 24 exposed subjects were determined and found to be diminished, again suggesting both primary and secondary testicular malfunction.

B. METHODS

Subjects were examined for testicular abnormalities. The study planners decided that the information should be available in the event that intergroup differences were found: differences in proportion of abnormalities among groups might then provide an alternate explanation to attribution to exposure.

Ejaculatory specimens were analyzed for sperm count and morphology. Each participating subject was asked to abstain from sex for at least 48 hours prior to producing a specimen into a polyethylene container and to bring the fresh specimen to the NIOSH facilities at the plant within 60-90 minutes after ejaculation. The specimens were labelled with identifying data plus the date and time the specimen was produced and the period of abstenance. Upon receipt, the specimens were observed for color, viscosity, and completion of liquification process. After measurement of specimen volume, it was frozen for transport back to the laboratory.

For analysis, each semen specimen was thawed completely and mixed well. Two dilutions were made using 5 percent bicarbonate - 1 percent formalin solution. Each dilution was counted twice and the results of 4 counts averaged. If a 20 percent discrepancy was found in any counts, repeat dilutions were made and a 5th and 6th count was made and averaged with the others. If a discrepancy was still present, duplicate tubes of 0.25 mg beta amylase in 0.01 ml 2.3 m $(NH_4)_2SO_4$ and 0.01 ml semen were mixed and allowed to sit for 30-60 minutes at room temperature. This mixture was then further diluted 1:5, 1:10, or 1:25 with distilled H_2O and the sperm counted twice per dilution. Values for all counts were averaged. All counts were made using a LEVY Hemocytometer Chamber. The final average count is the figure used for the statistical analysis.

At the same time the specimens were thawed and mixed for counting, several slides were smeared and fixed in 50 percent ethyl alcohol 50 percent ethyl ether solution for morphology studies. After 1-2 hours of fixation, the slides were rinsed with distilled H_2O, then manually stained by the Papanicolaou technique. The stained slides were evaluated under oil immersion (1000X) and 200 cells were counted. Based on the classification from MacLeod[3] each sperm was classified as either oval, large, small, tapering, amorphous, duplicate heads, duplicate tails, or spermatids. Counts for each individual were averaged and percentage figure of each cell type was recorded for statistical analysis.

Individual sperm counts will frequently have zero values for one or more of the abnormal sperm types. To analyze this multinomial data independently for each type, we would have to ignore the constraint that the sum was always 100% and might lose additional information. To circumvent this problem, we added the percent of each of the abnormal sperm forms and used this percent of abnormal sperm forms. Only "oval" forms were considered normal.

Komogorov's D-statistic was used to test the normality of data from the entire sample. Sperm count and volume, and the percent of abnormal forms were found to be log-normally distributed. This transformation was used in analyses.

Possible covariates, such as age associated with sperm count and volume, and sperm count associated with percent of any abnormal sperm forms were checked to see whether (1) they were significantly correlated to the respective variable in the expected direction, and (2) their slopes were parallel across groups. Age was significantly positively correlated to sperm count, but a negative correlation was expected. Sperm volume was not significantly correlated with age. Because of the uncertainty about how to deal with a covariate which correlated with the dependent variable in a direction opposite to what had been expected, both an analysis of variance and an analysis of covariance with age as a covariate were used to analyze transformed and untransformed sperm count data. Only analysis of variance was used to analyze sperm volume. Since a significant negative correlation was found between sperm count and the percent of any abnormal sperm forms (and between their normalized data), an analysis of covariance was used to analyze the data.

A regression analysis with either log sperm count or log sperm volume as dependent variable and age, log cumulative exposure index and their interaction as exploratory variables was performed.

Each subject was asked "Do you consider that your appetite for sex is below average, average, or above average?" and "Do you frequently have trouble getting or keeping an erection?" Responses were analyzed by current exposure level only.

C. RESULTS

Much of this information has been reported by Meyer; the methods used here differ only slightly from that report[5].

1. From Current Exposure

Table 11-1 shows demographic data for this subset of the study cohort (N = 154 total, Control N = 88, Exposed N = 66), including mean age, mean work duration, mean log(exp. ind.), and standard deviations for each. Table 11-2 shows the comparison between control and exposed groups for sperm counts, ejaculate volume, and percent abnormal forms. Differences between groups are not significant nor is there a consistent dose-related effect.

Table 11-3 compares responses to the question about sexual appetite (libido) among groups. Table 11-4 compares responses to the question about trouble getting or keeping an erection (potency) among groups. There are no significant differences.

2. From Cumulative Exposure

In regression analysis, the model for the log sperm count had a p-value equal to 0.03 and R^2 equal to 0.06, and that for the log sperm volume had a p-value equal to 0.16 and R^2 equal to 0.03. From the plots of the partial regression residuals, no apparent pattern was found. Thus, no further regression analysis was performed for sperm count and volume. A regression analysis with age, log sperm count, and log cumulative exposure index and product terms of the last variable and either of the first two variables as exploratory variables was performed on the transformed proportion of any abnormal sperm forms. The model has a p-value of 0.01 but the contribution of the product terms to R^2 was only 0.02. Another model without product terms was used. The multiple R^2 of the model was 0.09 with a p-value of 0.003. In this model log sperm count was the only significant variable ($p=0.0002$).

Since no differences were found among groups, analysis of testicular abnormalities was not carried out.

D. DISCUSSION

Statistical analysis of the sperm count, morphology and semen volume revealed no statistically significant difference between groups. The difference between the negative findings in this study and the positive findings in previous reports is probably related to the fact that this cohort had CS_2 exposures of 20 ppm or less, while the earlier groups were chronically intoxicated by CS_2. Thus it would seem from this study that exposures of 20 ppm or less do not cause diminished sperm count or abnormal sperm morphology. No significant differences in libido or in sexual pootency potency were detected by the two questions used in this study, suggesting that exposures of 20 ppm or less do not have adverse effects on these two variables.

E. REFERENCES:

1. Lancranjan I, Popescu HI, Kelpsch I: Changes of the gonadic function in chronic carbon disulfide poisoning. Med Lav 1969;60:566-71.

2. Lancranjan I: Alterations of spermatic liquid in patients chronically poisoned by carbon disulfide. Med Lav 1972;63:29-33.

3. MacLeod J, Hotchkiss RS: The Effect of Hyperpyrexia Upon Spermatozoa Counts in Men. Endocrinology 1941;28:780.

4. Maugeri S, Cavalleri A, Maugeri U, Taccola A: New Aspects in the Pathogenetic Mechanism of Carbon Disulfide Toxic Action. Med Lav 1971;62:398-403.

5. Meyer CR: Semen quality in workers exposed to carbon disulfide compared tyo a control group from the same plant. JOM 1981;23(6):435-439.

F. SUMMARY

Ejaculatory specimens were collected from 67 exposed and 89 comparison workers, frozen, and analyzed for sperm count and morphology upon return to the laboratory. Each subject was asked "Do you consider that your appetite for sex is below average, average, or above average?" and "Do you frequently have trouble getting or keeping an erection?" No significant differences were found among groups in sperm counts, ejaculate volume, morphologic characteristics, sexual appetite (libido), or difficulty getting or keeping an erection (potency).

Table 11-1: Demographic Characteristics of Exposed and Control Subgroups in Semen Analysis Participants

	N	Age [Years]		Employment Length [Months]		Log(Exp. Ind.) [Log(ppm-months)]	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Control	88	34.2	9.70	104.8	54.2	1.25	0.29
Exposed							
DL	21	38.3	10.3	140.1	89.7	2.64	0.48
M	27	38.4	8.8	158.2	59.7	2.92	0.28
DH	18	37.8	10.2	152.3	88.3	3.09	0.32
All Exposed	66	38.9	9.5	150.8	77.3	2.88	0.40
Test for Equality of Mean (by Welch)							
Control vs.							
Exposed		p = 0.01		p = 0.0001		p LT 0.0001	
DL vs. M vs. DH		p = 0.11		p = 0.0065		p LT 0.0001	
Test for Equality of Variance (by Levere's Test)							
Control vs.							
Exposed		p = 0.90		p = 0.0067		p = 0.02	
DL vs. M vs. DH		p = 0.61		p = 0.0065		p = 0.22	

Table 11-2: Sperm Count, Volume, and Frequency of Abnormal Sperm Forms by Current Exposure Level

	Control	DL	M	DH	All Exposed
Number	88	21	27	18	66
Sperm Count*					
- Mean	114.9	145.1	122.5	170.2	142.7
- SD	106.1	117.4	95.6	152.0	119.7
- Range	3-704	24-497	8-350	22-484	8-497
- Median	90.9	122.6	80	91.2	94.9
Log(Sperm Count)					
- Mean	7.89	8.02	7.95	8.05	8.00
- SD	0.42	0.39	0.38	1.86	0.39
Semen Volume					
- Mean(cc)	3.4	4.0	3.9	3.4	3.8
- SD(cc)	1.7	1.8	1.2	1.9	1.6
- Range(cc)	0.6-9.8	1.7-7.5	1.8-7.5	1.2-8.1	1.2-8.1
- Median(cc)	3	4	3.7	2.95	3.4
Log(Semen Volume)					
- Mean	0.47	0.56	0.57	0.48	0.54
- SD	0.24	0.20	0.14	0.22	0.18
Abnormal Forms (Not "oval")					
- Mean(%)	33.4	38.1	33.1	33.9	34.9
- SD(%)	13.5	16.0	12.6	11.4	13.4
- Range(%)	16-79	16-76	17-69	13-55	13-76
- Median(%)	31.5	35.0	29	33.5	30.5
- Adjusted⁺ Mean(%)	32.9	39.0	33.3	35.2	35.6
- Adjusted⁺ S.E.(%)	1.36	2.77	2.44	3.00	1.57

*All counts in millions/cc

⁺Mean adjusted for sperm count; S.E. is standard error of adjusted mean.

Table 11-3: Responses by Group to the Question "Do You Consider that Your Appetite for Sex is Below Average, Average, or Above Average?"

Number and (Percent)

	Below Average	Average	Above Average	Total
Control Group	6 (2.5)	194 (79.8)	43 (17.7)	243 (100.0)
Exposed Groups:				
DL	0 (0.0)	40 (81.6)	9 (18.4)	49 (100.0)
M	3 (4.6)	55 (84.6)	7 (10.8)	65 (100.0)
DH	3 (6.0)	39 (78.0)	8 (16.0)	50 (100.0)
All Exposed	6 (3.7)	134 (81.7)	24 (14.6)	164 (100.0)
Total	12 (3.0)	328 (80.6)	67 (16.5)	407 (100.0)

There are no significant differences among groups.

Table 11-4: Responses by Group to the Question "Do you frequently have trouble getting or keeping an erection?"

Number and (Percent)

	Yes	No	Total
Control Group	15 (6.2)	228 (93.8)	243 (100.0)
Exposed Groups:			
DL	2 (4.0)	47 (95.9)	49 (100.0)
M	1 (1.5)	64 (98.5)	65 (100.0)
DH	5 (10.0)	45 (90.0)	50 (100.0)
All Exposed	8 (4.9)	156 (95.1)	164 (100.0)
Total	23 (5.7)	384 (94.3)	407 (100.0)

There are no significant differences among groups.